Review Article

Emerging Harmful Algal Blooms and Human Health: *Pfiesteria* and related organisms

Lora E Fleming+*, Jana Easom+, Daniel Baden*+, Alan Rowan**, Bonnie Levin+.

*NIEHS Marine and Biomedical Sciences Center, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Miami, FL
+University of Miami School of Medicine, Miami, FL
**Division of Environmental Epidemiology, Florida Department of Health, Tallahassee, FL

Corresponding Author:

Lora E Fleming MD PhD MPH MSc
Associate Profession
Dept of Epidemiology and Public Health (R-669)
University of Miami School of Medicine
1801 NW 9th Avenue, Highland Park Building, Suite 200
Miami, FL
33136
<u>or</u>

PO Box 016069 (R-669)
Miami, FL
33101
tel: 305-243-5912
fax: 305-243-3384
email: lfleming@mednet.med.miami.edu

Key Words: Harmful Algal Blooms, Dinoflagellates, Marine Toxins, Estuarine Associated Syndrome, Red Tides, *Pfieisteria*

The opinions expressed in this article are those of the authors, not their employers or funding agencies.

The authors have all been involved in the spectrum of scientific issues surrounding the study of existing and emerging harmful algal blooms (HABs). Dr Fleming is an occupational and environmental health physician and epidemiologist; she is an Associate Professor in the Department of Epidemiology and Public Health of the University of Miami School of Medicine. Dr. Fleming is also the Director of Outreach and Education at the National Institute of Environmental Health Sciences (NIEHS) Marine and Freshwater Biomedical Sciences Center of the University of Miami Rosenstiel School of Marine and Atmospheric Science. She has worked on the human health issues of HABs for over 8 years with her colleague, Dr Daniel Baden, a biochemist who brings over 25 years of experience in the toxicologic aspects of HABs. Dr. Baden has served as the former Director of the NIEHS Marine Center at the University of Miami; he is now the Director of the Marine Center of the University of North Carolina at Wilmington. Ms. Jana Easom recently received her MPH at the University of Miami School of Medicine, after completing a study of individuals with occupational exposure to HABs in Florida; she will be attending medical school at the University of Miami School of Medicine. Mr. Alan Rowan is the Florida Department of Health HAB Coordinator, and has an extensive background in food safety issues; with Drs. Fleming and Baden, he is a member of the Florida HAB Taskforce. Dr Bonnie Levin is the Director of Neuropsychology at the University of Miami School of Medicine and the NIEHS Center; she works on the neuropsychologic effects of the HABs, as well as other issues.

Introduction

Algae are unicellular microscopic plant cells that are the foundation of life. An algal bloom develops in the marine or freshwater environment when there is an excess of growth of these organisms due to changes in that environment. A harmful algal bloom (HAB) is defined as a bloom that has deleterious effects on plants, animals and/or humans (1-5). HABs, such as red tides, have been occurring for centuries. In the recent years, they appear to be more frequent and extensive, both in the United States (US) and worldwide (1). In the US, the coasts have become the prime target of HABs. Some of the most affected areas are Florida, Maryland, Virginia, North Carolina, Louisiana, Texas, and Alaska. (1, 6).

The microscopic marine organisms that create the HABs (such as the dinoflagellates, cyanobacteria, and diatoms) are some of the most basic life forms on earth. They are found in freshwater and marine environments, as well as in the brackish estuarine systems. Some of the organims, often in the context of a HAB, produce toxins that may be harmful to other organisms. HABs can result in the death of fish, seabirds, and sea mammals. Humans usually are affected by HABs through the ingestion of contaminated seafood, although skin contact and inhalation of aerosolized contaminated water have also been implicated (7,8). With the emergence of newly discovered marine organisms, increased human-environment interactions, and a heightened awareness of the possibility of human health effects, there has been increased attention from the public health point of view. This has led to a flurry of research and funding focused on previously unrecognized HABs (6, 9-11).

Pfiesteria piscicida and the so-called "*Pfiesteria*-like" organisms were originally discovered in a laboratory setting, and then implicated in subsequent fish kills in the late 1980s and early 1990s (12). Investigators discovered the organism in some of the water and/or fish samples received from field biologists during events such as lesioned fish and fish kills occurring in North Carolina estuaries. Over the past several years, HABs in the mid Atlantic states have been associated with extensive fish kills, as well as multiple reports of a variety of human health effects associated with skin and aerosol exposure to reportedly HAB-contaminated water (13). Other *Pfiesteria*-like organisms have been implicated in fish events, in addition to *Pfiesteria piscicida*. For example, in February 1998, a new marine organism, a cryptoperidiniopsoid dinoflagellate resembling *Pfiesteria piscicida* morphologically and genetically, was identified in the estuarine waters of the St. Lucie River (St Lucie County, FL). This additional organism has been associated with fish lesions and has been identified in the Mid Atlantic fish events, often associated with *Pfiesteria piscicida*. No definitive human health effects have been reported associated with exposure to the waters of this river, although there has been considerable community and public health concern. To date, despite continued experiments, no toxin(s) have been isolated from either *Pfiesteria piscicida* or the *Pfiesteria*-like organisms (14-18).

The impacts of these new organisms on the environment and public health are yet to be resolved. There are reports that *Pfiesteria* may cause human health effects, fueling media alerts and focusing public attention on the issue (5, 10, 11, 19-24). Subsequently, pressure has been placed upon the research community to find definitive answers to the questions surrounding these organisms. This paper will evaluate the existing literature on *Pfiesteria piscicida* and the *Pfiesteria*-like organisms, focusing on possible human health effects, as well as delineate areas for further research.

Harmful Algal Blooms (HABs) Overview

Phytoplankton are unicellular organisms that float or swim freely in water such as dinoflagellates, cyanobacteria and diatoms. These organisms are microscopic, single-celled organisms that serve as the foundation of the food chain. Photosynthesis is the process that plant cells use to convert sunlight into energy by capturing light. Many of these organisms undergo photosynthesis and are a key link between sunlight and the process of creating life from energy. The dinoflagellates, cyanobacteria and diatoms are found throughout the world's oceans, estuaries and fresh waters (1, 7)

The life cycle of most HAB organisms is complex. One cell can go through various stages, some of which may be toxic and some which represent the organism in its dormant state. Typically, the first stage of the life cycle is an encysted form of the organism, resting dormant on the bottom of the ocean. As the conditions change, for example to warmer temperatures and stronger sunlight as well as other unknown factors, the cell leaves the cyst and becomes active, reproducing rapidly until the nutrient supply has diminished. This defines a "bloom." At this point, the cells undergo gamete formation and return to the encysted dormant state (7, 25).

When uncontrolled growth of these organisms occurs, the accumulation of dense patches results in a bloom or "red tide." These organisms, like the dinoflagellate associated with Florida Red Tide *Gymnodinium breve*, cause coloration of the waters (red, yellow, brown, green, or even white) during a bloom. Pigments within the organisms cause the color to appear on the surface of the water (25). Of note, these red tides and their ecological effects have been occurring for thousands of years (8, 9, 15, 25).

Of the several thousand species of dinoflagellates, cyanobacteria and diatoms, approximately 80 are known to be toxic (6, 7, 9). A red tide can be harmful to humans or fish when harmful toxins (often neurotoxins) are produced, although not all red tides have toxins. The toxins, small non-peptides, are some of the most powerful natural substances known; for example, the ciguatera fish poisoning toxin, ciguatoxin, is toxic to humans in a total body dose of 70 nanograms. Because these toxins are tasteless, odorless, and heat and acid stable, normal screening and food preparation procedures will not prevent intoxication if contaminated fish or shellfish is consumed (7, 26). When the toxins are present in high amounts as in a harmful algal bloom or HAB, the marine, estuarine and freshwater environments may be threatened, resulting in contaminated shellfish and fish, and the illness or death of fish, birds and even marine mammals and humans (8, 9).

In humans, the marine toxin diseases are categorized into two groups based on their primary transvectors. Shellfish harbor the toxins that produce paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrheic shellfish poisoning (DSP), and amnesic shellfish poisoning (ASP). Fish carry the toxins responsible for ciguatera poisoning and tetrodotoxin (fugu or pufferfish) poisoning. The shellfish-associated diseases generally occur in association with algal blooms or "red tides," which may be characterized by patches of discolored water and dead or dying fish. The fish-associated diseases are more localized to specific reef areas (ciguatera poisoning) and fish (fugu poisoning). There are also animal and human diseases associated with exposure to the blue green algae or cyanobacterial toxins and their blooms (7, 15, 27-30).

In addition to increased worldwide seafood consumption, other anthropogenic factors may have helped spread these organisms and their toxins. Human-assisted transportation of the dinoflagellates or their cysts occurs in spat cultivation (young bivalve shellfish sold commercially to global markets for aquaculture) and dumping of ship ballast water. In response, new international regulations will require ships to purge ballast water in the open ocean prior to docking (31). Human-generated environmental changes, such as reef destruction and eutrophication, also may help explain the apparent increase in human marine toxin disease as well as the increase in red tides reported worldwide. Global climate changes, which some suggest are linked to human activities, also may help explain the apparent global increase of algal blooms as well as the appearance of new marine toxin diseases like *Pfiesteria piscicida* (1, 2, 26, 32-34).

Pfiesteria piscicida

Pfiesteria piscicida was discovered inadvertently when cultured fish, tilapia, died after exposure to water collected from the Pamlico River in North Carolina in the late 1980s; it was first identified in the wild from water of the Albemarle-Pamlico estuary in 1991 (12, 35-37). Originally called "ambush predator dinoflagellate" to describe its behavior with fish prey (35, 37), *Pfiesteria* has been associated with fish ulceration disease and fish kills. In its toxic state, this organism can reportedly cause dermal ulcerations to fish, possibly making them less responsive to environmental stimuli. This invites bacteria, parasites and other predatory organisms to prey on the vulnerable fish (35. 37, 38). Since this time, researchers have worked to understand this organism by defining its geographic distribution and apparently complex life cycle, as well as the impact *P. piscicida* may have on the environment and human health. Most of the research concerning *P. piscicida* is still in its infancy, with a high the level of uncertainty surrounding the organism, its life cycle and biology, and its possible toxin(s) production.

One important issue surrounding the research of *Pfiesteria* concerns the problem and difficulty in identifying the organism. Since the discovery of *P. piscicida*, other dinoflagellates have been found which are morphologically similar, called "*Pfiesteria*-like organisms (PLOs)" or "morphylogically-related organims (MROs)." The organisms must be stripped of an outer membrane and viewed under electron microscope in order to accurately identify and distinguish between the various dinoflagellates (39). The difficulty in organism identification, which cannot be performed in the field, creates uncertainty with respect to earlier research and sampling results (36, 39, 40).

Life Cycle

The life cycle of *Pfiesteria piscicida* is reportedly extremely complicated, having 24 different unicellular stages of which the flagellated and amoeboid forms are the most significant. While dormant, *Pfiesteria* is an encysted amoeba, and is considered not harmful. Once the appropriate conditions are met, described below, the surrounding lipid soluble cyst is dropped, and the emerging cell can potentially cause problems. The most toxic is the "vegetative" stage that seems to be triggered by and multiplies in the presence of fish (12, 35-38, 41, 42).

Distribution

Pfiesteria appears to be most abundant in estuaries, regions where a river meets the ocean and the waters combine to create distinct ecosystem (43, 44). This marine environment contains relatively warm, brackish water with elevated nutrient levels and little wave action (38, 41, 42, 45, 46). Most of the affected fish have been found in calm, shallow, and poorly flushed areas, characteristic of estuarine environments (5).

First linked with a massive fish kill in North Carolina in 1991, *Pfiesteria* has been found on the East Coast of the United States from Delaware to Virginia. This organism has been associated with fish kills in the Chesapeake Bay and other estuarine/river systems in Maryland. One of the key issues surrounding *P. piscicida* is the presence of other organisms during fish events. Other dinoflagellates including the *Pfiesteria*-like organisms may be competing or co-causal factors of the fish kills. It is difficult to distinguish between many of these due to the complex identification procedure described above to properly identify each organism (12, 15, 38, 39, 41, 42).

Effects on Fish

Since its discovery, *Pfiesteria* has been implicated in various fish-related events over the past decade, yet there have been fish kills and lesioned fish present in estuarine waters for many years, often lasting many weeks. In the laboratory, fish kills apparently caused by *Pfiesteria* are short-lived; the toxic effects last only for a few hours, after which the organism allegedly reverts to its non-toxic state. The level of toxicity is apparently measured as the number of *P. piscicida* in the "toxic" amoeba stage (12, 38, 41, 42, 47). In a laboratory that attempted to simulate the conditions of the estuarine environments, the effects of *Pfiesteria* on fish have been tested. Once the fish were removed from experimental aquariums, the levels of *Pfiesteria* in the alleged toxic state declined. In the areas with fish kills that have tested positive for *Pfiesteria*, once the fish are removed, there appeared to be a loss of toxicity (ie. number of *P. piscicida*) within 24 hours (12, 37).

Ulcerated Disease Syndrome (UDS) has been noted in fish since the 1970s; UDS presents with fish lesions through the deterioration of fish skin. With the discovery of the new marine organism, UDS has now been associated with *P. piscicida* and other *Pfiesteria*-like organisms. It is suspected that fish secretions (such as excrement) stimulate the growth of the dinoflagellates, causing a bloom.). Fish have been reportedly noted to develop other symptoms due to *Pfiesteria* such as decrease in feeding habits and a loss of instinctive response to predators and danger (38, 41, 42, 45, 46). It is unclear whether *P. piscicida* and/or the PLOs actually cause the fish lesions, or if they cause lethargy among fish allowing for other opportunistic microorganisms such as bacteria and/or fungi to prey upon the susceptible fish. In addition to problems with the identification of the organism(s) and toxin(s), there is uncertainty concerning the temporal factors, what causes these lesions specifically, and what triggers each event (15).

Environmental Conditions

There are several conditions that are suspected to be conducive for the proliferation of the toxic form of *P. piscicida*. However, it remains uncertain what actually causes the dinoflagellate to change from its non-toxic stage, triggering the apparent toxic behavior and "attack" on fish (5, 12).

Fish kills have occurred frequently along the East Coast of the US for centuries. The majority of fish kills during the 1980s were attributed to low amounts of dissolved oxygen. Due to limited levels of available oxygen and increased nutrient composition, large numbers of fish died as a result of incompatible living conditions. Other possible explanations include changes in the chemical balance of the marine environment that influence the microscopic community. Nitrogen and phosphorus are two of the most important nutrients, and the shifting ratio of the two alters the composition of coastal waters. Phosphorus has been increasing, and there is a possible hypothetical direct relationship between the amount of both phosphorus and dinoflagellates. As a result, *Pfiesteria* is alleged to thrive in the excess of this element. However, the true relationship between these elemental factors and the presence of *Pfiesteria* has not been causally defined (5, 12, 15, 38).

One of the popular explanations of the latest surge in dinoflagellates in general and *Pfiesteria* itself is the effects of humans on the environment. Human activities may be responsible for elevated bacterial counts due to wastewater solid effluent from agricultural facilities, wastewater treatment plants, and other high nutrient activities. These bacteria serve as a vital food source for the dinoflagellates and fish that in turn are eaten by *P. piscicida*, promoting rapid growth. Although these problems are believed to be factors, there has been no substantiated evidence that reducing the phosphorus count and/or nutrient load will conclusively eliminate or even decrease the fish events and level of *Pfiesteria* in the waters (5, 15).

Pfiesteria piscicida Toxin(s)
As stated earlier, no toxin(s) associated with *Pfiesteria* have been isolated. In a series of small experiments, Levin et al (1997) injected albino Sprague-Dawley rats subcutaniously with aquarium water provided by Burkholder's laboratory (48). Reportedly recently collected samples (transported frozen) were associated with learning impairment, specifically for learning new tasks, as well as deficits in habituation of arousal and rearing behavior in a functional battery, lasting up to 10 weeks from injection. A single observer was reportedly blind to exposure status. No other effects were noted on the hematologic or pathologic examinations. There is no characterization of the injected aquarium water beyond ammonia and nitrate levels with *Pfiesteria* cell counts from reportedly fish-lethal cultures. The small sample size, issue of mixed substances in the injections, and mixed results of the statistical testing make this study inconclusive but hypothesis generating.

Research is underway which seeks to identify toxin(s) (as well as what may stimulate toxic transformation) that may be produced by this dinoflagellate. The discovery of toxin(s) would provide the necessary causal link between the fish events and the organism. The research community is reportedly searching for at least three toxins: two lipid-soluble and one water-soluble, to date without success. One of the lipid-soluble toxins (dermonecrotic) may cause fish ulcerations, the other one is labeled the lipid-soluble lethal factor (LSLF); the water-soluble toxin may have possible mechanisms involving the presumed neurotoxic effects on humans (5). Discovery of the toxin(s) could lead to necessary dose-response relationships and biological mechanisms needed to determine possible human health effects. Without the definitive presence of a toxin, much of the reported effects can only remain suspect until validation and discovery of a specific marine toxin is proven to be produced by *Pfiesteria*. Furthermore, evaluation of possible human health effects is hampered by the lack of identifiable toxin and biomarkers of exposure (5, 15, 40).

Epidemiological Studies

There have been several studies investigating the possible health effects of *Pfiesteria* exposure on humans. These studies have focused on people who were potentially exposed occupationally and recreationally during events and/or laboratory work predominantly in North Carolina and Maryland.

When evaluating the epidemiologic research in this area, there are a several issues of epidemiological concern (5, 40). The basic science research necessary to study these organisms is just beginning. At present, there exists no easily available method of identifying the *Pfiesteria piscicida* organism and the *Pfiesteria*-like organisms, especially in the field. Furthermore, as discussed above, the presumed toxin(s) associated with these organisms have not been identified. Therefore, there are presently no useful measures of exposure for epidemiologic or clinical studies. Instead, exposure has been defined as self-reported occupational and/or recreational exposure to estuarine waters by skin and/or aerosol route (13). Furthermore, in part hampered by the lack of exposure measures, the measures of possible human health effects have tended to be predominantly self-report of a wide variety of symptoms without objective evidence of effect (13, 17, 40). Without appropriate exposure and effect measures, it is difficult to accurately define the possible human health effects associated with exposure to these organisms. Therefore, the epidemiologic research of the *Pfiesteria piscicida* organism and the *Pfiesteria*-like organisms is its infancy and should be interpreted as such.

Initial Human Case Reports
A case series of 10 individuals (reported by these same individuals) working in various laboratory settings associated with *Pfiesteria* research was analyzed for illness due to possible aerosolized and dermal exposure aquarium water where *Pfiesteria* organisms were being studied (38). Cultures of the organism were grown

to mimic natural water conditions to determine basic information regarding life and reproductive cycles. Exposure to this organism and the presumed aerosolized toxins were defined as working with cultures of *Pfiesteria* for 1-2 hours over several months, although two of the individuals were reportedly exposed by occupying offices near the culture area (38).

The laboratory workers reported multiple and varied symptoms (including sensory disturbances, stomach cramping, memory loss, respiratory irritation, emotional lability, etc.) for varying degrees of time associated with occupational exposure to the aquarium water and its aerosol. The symptoms, especially the short term memory loss, reportedly lasted for weeks to months; in general, elimination of exposure resulted in the resolution of the symptoms. The investigators associated the degree of exposure with the length of time the individual was symptomatic, particularly the memory loss. Of note, two persons reported symptom resolution after using face masks/ventilators. One person was believed to be "modeling" since symptoms were only reported in the presence of another individual with reporting eye irritation. Unfortunately, systematic testing was not carried out on these individuals, and their improvement is based solely on anecdotal report.

Considerable but varied medical evaluation was carried out on a subgroup of the workers, including neurologic examination, routine blood hematology and chemistries, a toxicology screen for heavy metals, EMG/NCV, spinal fluid evaluation, brainstem and visual evoked potentials, MRI, PET scan, and neuropsychologic testing. The majority of these tests were within normal limits or inconclusive. Only one person apparently received neuropsychologic testing, with results reported as a definite organic syndrome with amnestic syndrome involving verbal more than visual disturbances (12, 38).

The reported memory loss and other neuropsychological symptoms from possible aerosolized and dermal exposures to *Pfiesteria* organisms were taken seriously by members of the scientific community because of prior studies documenting irreversible memory loss among individuals exposed to another marine toxin, domoic acid (49). In addition, aerosolized brevetoxin exposure from the Florida Red Tide had been associated with reported human health effects (8). The Glasgow et al (1995) case series represents the first reported cases of possible human health effects from apparent occupational exposure to *Pfiesteria* organisms. No *a priori* strict case definition with objective health effects criteria was applied to determine the cases and the non-cases. The people who were cases were categorized based on their self-report of symptoms and work exposure. Exposure itself was loosely defined based on job title and physical location; there was no objective measure of exposure, such as levels of *Pfiesteria* or toxin(s). The entire denominator of potentially exposed persons was not evaluated. Thus, the case definition consisted of an extremely broad definition of symptoms and possibly associated diseases, without consistent objective measures and with a vague exposure definition.

Further Case Series

A prolonged fish kill with fish with lesions occurred in the Chesapeake Bay and Pocomoke River estuary along the eastern shore of Maryland, starting in the Fall of 1996 and augmenting through the summer of 1997. During the summer of 1997, *Pfiesteria* organisms and *Pfiesteria*-like organisms were reported by Burkholder and her colleagues in water sample analyses (50). This resulted in a flurry of human health research activity, as well as the temporary closure of the Maryland waterways to commercial activity.

A case series was reported by a local physician (51, 52). These patients reported experiencing several different symptoms such as rashes, headaches, diarrhea, and memory loss. Some of the cases had skin lesions or diarrhea only; others burning sensations and/or memory loss; while still others reported a variety of symptoms including those mentioned, in addition to dizziness, nausea and shortness of breath. The onset of these symptoms ranged from a few days to months after the fish kills. Reportedly psychometric testing done on an unspecified subset of patients revealed the "fingerprint of abnormalities seen with *Pfiesteria* exposure." Although labeled a "syndrome," there was no consistent symptom complex apparent to link all of these patients together. There were no objective measures of exposure. The cases reported recreational and/or occupational exposure to river water in the area of the fish kills or to killed fish. Allegedly the cholesterol binding agent, cholestyramine, provided relief from symptoms in a subset of cases; however, the drug was administered in a non-blinded, non-random fashion (51, 52).

A group of Maryland investigators collaborated to evaluate individuals with predominantly occupational exposure to estuarine waters with fish kills in the Chesapeake Bay status (50). Initially 13 persons were examined, but ultimately the study included 24 participants with variable exposure primarily through their occupation in large fish kills in August of 1997 and earlier. In this study, participants were asked for information regarding medical history, symptoms, and 13 were given a medical evaluation and lab analysis. A battery of neuropsychological tests was given to determine effects in the individuals. These 24 people were compared to a control group of reportedly similar age, sex, education and occupation; this control group was assembled after the original evaluation. Alleged human health effects were evaluated by exploring cases (complaining of symptoms) who ranged from lab workers to fisherman, all of whom were involved in some aspect of the clean-up and investigation of the fish kills. Analysis of possible human health effects in the study population (5 excluded, 19 remaining) was based on neuropsychological tests that measured learning and memory capabilities (Rey AVLT), attention (Stroop Color-Word Test, Trails), and motor skills (Grooved Pegboard task), in addition to lab tests, dermatological exams, and other neuropsychological tests. The medical examination of the subgroup included pulmonary function tests and skin biopsies if necessary, and laboratory tests (50)

The exposure definition was based on the reported activities the individual participated in during a fish kill with presumed exposure to *Pfiesteria* as a surrogate for exposure to the alleged toxin(s). The possible routes of exposure were through variable inhalation and/or skin contact with contaminated water. There were varied reported estuarine water exposures, ranging from "high" (defined as 6-8 hours/day) to "moderate" (8-20 hours/week) to "low" with little or no direct skin contact (<40 hours total); the job categories ranged from "watermen" to government environmental workers. The highly exposed group consisted of 7 out of 8 watermen who worked in the effected estuaries. Reportedly the majority of the participants were evaluated within 2 weeks from their last exposure to estuarine waters. Two of the 24 exposed subjects were excluded due to incomplete symptom data.

The investigators reported that the cases were more likely to complain of neurological problems than the controls, although there was no definitive difference between the exposed and controls groups with either the medical or lab exams. The exposed group was noted to have lower scores on the some of the neuropsychological exams compared to the controls. There was a reported difference in one of five parts of the learning tests between the four groups, with the highly exposed group reportedly learning significantly fewer words. A linear response was noted, indicating that subjects with the reported highest exposure had the lowest performance on the Stroop Color-Word Test, AVLT, and Grooved Pegboard task (50). investigators were not blinded to the exposure status of all participants, although statistical analysis reportedly found no difference.

During follow-up examinations, researchers found the test scores on part of the Rey AVLT improved, interpreted by the investigators as a reversal of the effects of the possible toxic exposure. There were no differences among the other exams, including dermatologic. The researchers concluded that the effects of *Pfiesteria* and the presumed toxins can be eliminated by removing the exposure (50). Of note, the follow-up exams were only performed on 10 of the original exposed participants and 12 of the original control group individuals in the study.

There are a number of epidemiologic concerns raised by this study. The original study group was a case series of individuals self-defined in terms of their exposure and health effects labeled “cases” and “controls”; the additional cases and “control group” were assembled later. No *apriori* hypotheses were formulated. In addition, no objective measures of exposure other than self-report of estuarine water contact were used to categorize subjects into the study subpopulations. The actual study numbers are very small, especially with the exclusion of cases and the subsequent incomplete follow up; therefore, the power of the study to detect a true significant difference is very small. This is especially true when there is the application of multiple statistical tests as is necessary when analyzing the large battery of neuropsychological tests administered by the investigators. Further concerns intrinsic to neuropsychological testing include significant effects of confounding from education and socio-economic class, especially in the setting of significant anxiety and lack of baseline information for different population subgroups (17, 18). Given these limitations, Grattan et al (1998a) study is limited in its generalizability and should be viewed as a hypothesis-generating pilot study (50).

Other human health effect related studies

Many of the same Maryland investigators published a series of articles directed at physicians on the possible human health effects and other aspects of *Pfiesteria* (53-65). In general, these articles represent further publication of the already discussed articles by Grattan et al (1998a), Burkholder et al (1997) and Glasgow et al (1995) (12, 38, 50). One study by Golub et al (1998) attempted to use participants in a toll free hotline to evaluate whether consumption of seafood was associated with self-reported *Pfiesteria* exposure and a low score on the neuropsychological test described above, the Rey Auditory Verbal Learning Test (61). Reportedly, although associated with fishing and handling fish with lesions, a decreased score was not associated with seafood consumption.

As a part of a North Carolina study, Hudnell (1998) investigated the association between fish kills reportedly caused by *Pfiesteria piscicida* and the effects on vision (as another neurologic indicator) (66). Twenty-two people were studied in the “exposed” group, defined as individuals who worked in estuaries where *Pfiesteria* was present. The “unexposed” group, matched for age, gender, and education, although not for occupation, consisted of 20 individuals who worked offshore but lived in estuarine areas. The vision testing involved a visual acuity test for normalization and the Visual Contrast Sensitivity (VCS) test. The VCS analyzes the ability to distinguish between light and dark patterns; VCS has been used to detect neurotoxic effects in adults with other exposures such as solvents. These tests were administered to both groups during the battery of tests in a larger case series study. Hudnell found that there was no significant difference between the two groups for the visual acuity test, however there was reportedly a difference in the VCS test indicating that the “exposed” group exhibited lower scores in pattern detection. Further analysis excluding subjects of both groups with a history of neuropsychological impairment and excluding those without direct fish kill exposure continued to show a VCS deficit. As with Grattan et al (1998a), although possibly hypothesis generating, this study cannot be considered conclusive of neuropsychological deficits associated with *Pfiesteria* exposure (50). In particular, there is a lack of objective exposure measurement (with possible exposure misclassification) and unexposed baselines, as well as statistical issues associated with multiple tests with a small study population.

CDC/States Workshop Definition and Current research

In September 1997, the Centers for Disease Control and Prevention (CDC) held a “Public Health Response to *Pfiesteria* Workshop” in order to coordinate response teams for states affected by these and other estuarine organisms (13). This meeting and the resulting definitions were created jointly in an effort to bring uniformity to the research and evaluation of fish events. Definitions for environmental exposure to *Pfiesteria* or morphologically related organisms (MROs) (also known as "Pfiesteria-like"), with subsequent modifications over time, are as follows:

EXPOSURE DEFINITION

- Fish with lesions consistent with *P. piscicida* or MRO toxicity (20% of a sample of at least 50 fish); or
- A fish kill involving fish with lesions consistent with *P. piscicida* or MRO toxicity; or
- A fish kill involving fish without lesions, if *P. piscicida* or MRO's are present and there is no alternative reason for the fish kill.

The cluster of human health effects that have been defined as signs and symptoms persisting at least 2 weeks or more (except for skin rash or burning) with exposure to *Pfiesteria* or MROs are:

SIGNS AND SYMPTOMS DEFINITION

- memory loss, or
- confusion, or
- three or more of an additional set of conditions: headaches, skin rash at site of water contact, sensation of burning skin, eye irritation, upper respiratory irritation, muscle cramps, and gastrointestinal complaints; and,
- A healthcare provider cannot identify another cause for these symptoms.

Given the high degree of exposure uncertainty, the above disease and exposure description was labeled "Estuarine Associated Syndrome (EAS)." Under the direction and funding of the CDC, several states began investigating the human health effects of *Pfiesteria* and other estuarine organisms using EAS as a surveillance tool.

Due to the lack of identification of a specific organism, the similarity of *Pfiesteria* to other dinoflagellates (PLOs and MROs), the lack of resources to accurately sample alleged exposures, and the absence of identified toxin(s), the CDC/States definition of a case of EAS was further broadened to encompass all individuals who are exposed in estuaries with similar symptoms. Researchers established "Possible Estuarine Associated Syndrome (PEAS)" as the name for the illness (with the same qualifying symptoms as EAS) reported after exposure to *any* estuarine environments, even those without the confirmed presence of *Pfiesteria* or MROs. Such ambiguity reflects the uncertainty that the fish events and reported human health effects are due to *Pfiesteria* alone, other dinoflagellates, or something else entirely.

As per the CDC/States protocol for PEAS/EAS surveillance, the PEAS cases undergo an initial interview to see if they meet the symptom and exposure criteria. For the purposes of exposure, the criteria become less stringent. The individual must only report having been exposed to an estuary (regardless of presence of fish kills, lesions or *Pfiesteria*), in addition to appropriate symptoms. For example in Florida, the South Florida Poison Control Center and a medical response team from the University of Miami are investigating individuals suspected of having both exposure and symptoms that are reportedly associated with exposure to Florida estuarine waters. In addition, efforts were begun in several mid-Atlantic states to assemble "cohorts" (really non-randomly selected subpopulations) of possibly estuarine and HAB exposed individuals to monitor for possibly human health effects over time.

Conclusions

Harmful algal blooms appear to be increasing worldwide with the potential for increasing impact on human health. International travel and trade, increased seafood consumption, and increased interaction of large numbers of humans with the marine and estuarine environments mean increased potential exposure of humans to the organisms and their blooms. *Pfiesteria* and the *Pfiesteria*-like organisms are one of several groups of newly identified organisms with potentially toxic effects on human health and the environment.

To date, there is a small body of suggestive but necessarily inconclusive scientific research in the area of possible human health effects resulting from exposure to *Pfiesteria* and the *Pfiesteria*-like organisms. As has been stated above, without objective and easily applicable measures of exposure, as well as a lack of identifiable toxin(s), it is very difficult to perform definitive epidemiologic studies of the possible human health effects of *Pfiesteria* and the *Pfiesteria*-like organisms. This situation is further complicated by the apparent necessity of using batteries of neuropsychologic tests as the objective measure of human health effect, as well as self-report of a large range of symptoms (as seen in the P/EAS case definition). Neuropsychologic testing is best performed on relatively large populations with pre-exposure baselines and/or with carefully matched controls in terms of education, socio-economic class, and other possible confounders such as alcohol exposure.

In the future, research on *Pfiesteria* and the *Pfiesteria*-like organisms must begin to focus on accurately defining the organisms, finding and characterizing the presumed toxins, evaluating their environmental effects, developing objective measures of exposure and human health effects, and determining the specific mechanisms, both environmental and biological, that lead to possible human illness. Longitudinal studies of human populations, such as currently initiated by the CDC, will enable the development of a more epidemiologically sound data on which to base the assumed impact of these organisms on human health, although efforts must be made to avoid self-selection of these cohorts (5, 17, 40, 67). In the meantime, from the public health point of view, these and other data suggest that increased care should be exercised by individuals with possible occupational exposures to HABs, both in the laboratory and the field.

References

1. Epstein P (1998). *Marine ecosystems: emerging diseases as indicators of change*. Boston, MA: Harvard Health Ecological and Economic Dimensions (HEED) of Global Change Program.

2. Todd ECD (1994). Emerging diseases associated with seafood toxins and other water-borne agents. *Annals N.Y. Acad. Sci*. 740:77-94.

3. Anderson DA (1994). Red tides. *Sci. Am.* 271:62-68.

4. Epstein P (1995). Emerging diseases and ecosystem instability: new threats to public health. *Am. J. Public Health*. 85: 168-172.

5. Anonymous (1998). *The Raleigh Report 1998: Pfiesteria Research Needs and Management Actions*. Research Triangle Park, NC: Water Resources Research Institute of the University of North Carolina.

6. ECOHAB (1998) *What is a HAB?* www.fmri.usf.edu.

7. Baden D, Fleming L, Bean JA (1995). Marine Toxins. In *Handbook of Clinical Neurology: Intoxications of the Nervous System Part II. Natural Toxins and Drugs*. F. A. deWolff, F., ed. Elsevier Press, Amsterdam, Netherlands, pp. 141-175.

8. Fleming LE, Baden DG (1998). *Neurotoxic Shellfish Poisoning: Public Health and Human Health Effects*. White Paper for the Proceedings of the Texas Conference on Neurotoxic Shellfish Poisoning, Proceedings of the Texas NSP Conference, Corpus Christi (Texas), pp. 27-34.

9. Mote Marine Laboratory website (July 1998). *About Red Tide*. www.mote.org/~mhenry/REDTIDE.phtml.

10. Tibbetts J (1998). Toxic Tides. *Env. Health Persp.* 106(7): A326-A331.

11. Pelley J (1998). What is causing toxic algal blooms? *Env. Sci. Tech. News*. 32(1):26-30.

12. Burkholder JM, Glasgow HB, Lewitus AJ (1997). Physiological ecology of Pfiesteria piscicida with general comments on "ambush predator" dinoflagellates. In: *The Physiological Ecology of Harmful Algae*. Anderson DM, Cembella A, Hallegraef EM, eds. NATO Workshop Proceedings, *in press*.

13. Centers for Disease Control and Prevention (CDC)(1997) . Results of the public health responsde to the *Pfiesteria* workshop -- Atlanta, Georgia, Sept 29-30, 1997. *Morb. Mort. Weekly Rep*. 46:951-952.

14. Fleming LE, Easom J (March 1999). *Harmful Algal Blooms Screening Pilot Study*. Miami, FL: Report for the Florida Dept of Health.

15. Steidinger KA, Landsberg JH, Tomas CR, Burns JW (March 1999). *Harmful Algal Blooms in Florida*. St Petersberg, FL: Florida Dept of Environmental Protection Harmful Algal Bloom Task Force.

16. Landsberg JH, Steidinger KA, Blakesley BA (1995). Fish killing dinoflagellates in a tropical marine aquarium. In: *Harmful Marine Algal Blooms*. Lassus P, Arsul G, Erard E, Gentien P, Marcaillou C eds.

Paris: Lavoisier Intercept Ltd, pp. 65-70.

17. Smith CG, Music SI (1998). *Pfiesteria* in North Carolina: the medical inquiry continues. *N. Carolina Med. J.* 59(4):216-220.

18. Rullan JV, Jenkins SR (1998). The public health implications of *Pfiesteria* in Virginia. *Virginia Med. Quart.* 125(1):78-80

19. Anonymous (1998). New source of fish fears. *Env. Health Persp*. 106(9): A425-A426.

20. Shoemaker RC (1998). *Pfiesteria*! Crossing Dark Water. Baltimore, MD: Gateway Press.

21. Kaiser J (1997). New clues for two toxicological mysteries. *Science*. 276:201.

22. MacIlwain C (1997). Scientists close in on "cell from hell" lurking in Chesapeake Bay. *Nature*. 389:317-318.

23. Semans S. *Pfiesteria* (3 Part Series) (Sept 2-23, 1998). Pamlico, NC: *The Pamlico News*.

24. Barker R (1997). *And the Waters Turned to Blood: the Ultimate Biological Threat*. New York: Simon and Schuster.

25. Tester PA, Steidinger KA (1997). *Gymnodinium breve* red tide blooms: initiation, transport, and consequences of surface circulation. *Limnol. Oceanogr*. 42 (5, part 2):1039-1051.

26. Halstead BW (1994). Other poisonous marine animals. In *Foodborne Disease Handbook*. Y. H. Hui, J. R. Gorham, K. D. Murrell, D. O. Cliver, eds. Marcel Dekker Inc, New York, pp.. 497-528.

27. Carmichael WW (1994). The toxins of cyanobacteria. *Scientific Am. Jan*. 78-86.

28. Falconer IR (1999). An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. *Env. Toxicol.* 14:5-12.

29. Fleming LE, Easom J (1998). Seafood Poisonings. *Travel Medicine*. 2 (10):1-8.

30. Fleming, L.E., Baden, D.G., Bean, J.A., Weisman, R., and Blythe, D.G (1998). Seafood toxin diseases: Issues in Epidemiology and community outreach. In: *Harmful Algae* (B. Reguera, J. Blanco, M.L. Fernandez, and T. Wyatt, Eds.) Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, pp. 245-248.

31. Hallegraeff GM, Bolch CJ (1992). Transport of diatom and dinoflagellate resting spores in ships' ballast water: implications for plankton biogeography and aquaculture. *J. Plankton Res*. 14(8):1067-1084.

32. Viviani R (1992). Eutrophication, marine biotoxins, human health. *Sci. Total Environ. - Supplement.* 631-62.

33. Tester PA (1994). Harmful marine phytoplankton and shellfish toxicity. *Annals N.Y. Acad. Sci*.

740:69-77.

34. Epstein PR, Ford TE, Colwell RR (1994). Marine ecosytems. In: *Health and Climate Change*. P.R. Epstein, D. Sharp, eds. The Lancet Ltd, London. pp. 14-17.

35. Burkholder JA, Noga E, Hobbs C, Glasgow H (1992). New phantom dinoflagellate is the causative agent of major estuarine fish kills. *Nature.* 358;407-410.

36. Smith SA, Noga EJ, Bullis RA (1998). Mortality in *Tilapia aurea* due to a toxic dinoflagellate bloom (abstract). *Proc 3rd International Colloquium Path Mar Aquaculture, Gloucester*. pp. 167-168.

37. Steidinger KA, Burkholder JM, Glasgow HB, Hobbs CW, Garrett JK, Truby EW, Noga EJ, Smith SA(1996). *Pfiesteria piscicida* gen. et sp. nov. (Pfiesteriaceae, fam. nov.), a new toxic dinoflagellate with a complex life cycle and behavior. *J. Phycology*. 32:157-164.

38. Glasgow HB, Burkholder JM, Schmechel DE, Tester PA, Rublee PA (1995). Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. *J. Tox. Env. Health.* 46:501-522.

39. Steidinger KA, Landsberg JH, Truby EW, Blakesley BA (1996). The use of scanning electron microscopy in identifying small "gymnodiniod" dinflagellates. *Nova Hedwigia*. 112:415-422.

40. Fleming LE, Bean JA, Baden DG (1995). Epidemiology of Toxic Marine Phytoplankton. In *UNESCO-IOC Manual on Harmful Marine Phytoplankton #33.* G. M. Hallegraeff, D.A.N. Anderson, A.D. Cembella, eds. UNESCO, Paris, pp. 475-488.

41. Burkholder JM, Glasgow HB, Hobbs CW(1995). Fish kills linked to a toxic ambush predator dinoflagellate: distribution and environmental conditions. *Mar. Ecol. Prog. Ser*. 124:43-61.

42. Burkholder JM, Glasgow HB (1995). Interactions of a toxic estuarine dinoflagellate with microbial predators and prey. *Arch. Protistenkd*. 145:177-188.

43. National Estuary Program (June 1998). *Estuary Overview*. www.epa.gov/region04/waterpgs/water/coastal/nepover.html.

44. Lewitus AJ, Jesien RV, Kana TM, Burkholder JM, Glasgow HB, May E (1995). Discovery of the phantom dinoflagellate in Chesapeake Bay. *Estuaries*. 18(2):373-378.

45. Burkholder JM, Glasgow HB (1997). Trophic controls on stage transformations of a toxic ambush predator dinoflagellate. *J. Eukary. Microbiol*. 44(3):200-205.

46. Burkholder, JM (1997). *Pfiesteria piscicida* and other toxic *Pfiesteria*-like dinoflagellates: behavior, impacts and environmental controls. *Limnology Oceanography.* 42(5 part 2): 1052-1075.

47. Environmental Protection Agency (EPA) (1998). *What you should know about Pfiesteria piscicida*. Washington, DC: US Government Printing Office Document #EPA 842-F-98-011.

48. Levin ED, Schmechel DE, Burkholder JM, Glasgow HB, Deamer-Melia NJ, Moser, VC, Harry GJ (1997). Persisting learning deficits in rats after exposure to *Pfiesteria piscicida*. *Env. Health*

Perspectives. 105(12):1320-1325.

49. Perl TM, Bedard L, Kosatsky T, Hockin JC, Todd ECD, Remis R (1990). Encephalopathy Caused by Contaminated Mussels. *New Eng. Med. J*. 322: 1775-80.

50. Grattan LM, Oldach D, Perl TM, Lowitt MH, Matuszak DL, Dickson C, Parrott C, Shoemaker RC, Kauffman CL, Wasserman MP, Hebel JR, Charache P, Morris JG (1998). Learning and memory difficulties after environmental exposure to waterways containing toxin-producing *Pfiesteria* or *Pfiesteria*-like dinoflagellates. *Lancet*. 352:532-539.

51. Shoemaker RC (1997). Diagnosis of *Pfiesteria* human illness syndrome. *Maryland Med. J.* 46(10):521-523.

52. Shoemaker R (1998). Treatment of persistent *Pfiesteria* human illness syndrome. *Maryland Med. J.* 47(2):64-66.

53. Morris JG (1998). *Pfiesteria*! An introduction. *Maryland Med. J.* 47(3):104-105.

54. Kane AS, Oldach D, Reimschuessel R (1998). Fish lesions in the Chesapeake Bay: *Pfiesteria*-like dinoflagellates and other etiologies. *Maryland Med. J.* 47(3):106-112.

55. Oldach D, Brown E, Rublee P (1998). Strategies for environmental monitoring of toxin-producing phantom dinoflagellates in the Chesapeake. *Maryland Med. J.* 1998;47(3):113-119.

56. Bever CT, Grattan L, Morris GJ (1998). Neurologic symptoms following *Pfiesteria* exposure: case report and literature review. *Maryland Med. J*. 47(3):120-123.

57. Lowitt MH, Kauffman CL (1998). *Pfiesteria* and the skin: a practical update for the clinician. *Maryland Med. J.* 47(3):124-126.

58. Grattan LM (1998). Current status and future directions for the investigation and management of the human health effects of exposure to *Pfiesteria piscicida* or *Pfiesteria*-like dinoflagellates. *Maryland Med. J.* 47(3):148-150.

59. Tracy JK, Oldach D, Greenberg DR, Grattan LM (1998). Psychologic adjustments of watermen with exposure to *Pfiesteria piscicida*. *Maryland Med. J.* 47(3):130-132.

60. Greenberg DR, Tracy K, Grattan LM (1998). A critical review of the *Pfiesteria* hysteria hypothesis. *Maryland Med. J.* 47(3):133-136.

61. Golub JE, Haselow DT, Hageman JC, Lopez AS, Oldach DW, Grattan LM, Perl TM (1998). *Pfiesteria* in Maryland: preliminary epidemiologic findings. *Maryland Med. J.* 47(3):137-143.

62. Matuszak DL, Taylor JL, Dickson C, Benjamin GC (1998). Toxic *Pfiesteria* -- surveillance for human disease in Maryland. *Maryland Med. J.* 47(3):144-147.

63. Grattan LM, Oldach D, Tracy K, Greenberg DR (1998). Neurobehavioral complaints of symptomatic persons exposed to *Pfeisteria piscicida* or morphologically related organisms. *Maryland Med. J.*

47(3):127-129.

64. Matuszak DL, Sanders M, Taylor JL, Wasserman MP (1997). Toxic *Pfiesteria* and human health. *Maryland Med*. J. 46:515-520.

65. Lutz LL, Burnett JW (1998). Pfiesteria piscicida. *Cutis.* 61(2):122.

66. Hudnell K (August 1998). *Human Visual Function in the North Carolina Clinical Study on Pfiesteria Piscicida.* RTP, NC: US Environmental Protection Agency.

67. Hulka BS, Griffith JD, Wilcosky TC, eds (1990). *Biological Markers in Epidemiology*. Oxford University Press, New York.