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C Rubin
et al

Eugene M. Burreson
Virginia Institute of Marine Science

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Emerging Areas of Research Reported during the CDC National Conference on *Pfiesteria*: From Biology to Public Health

Carol Rubin,[1] Michael A. McGeehin,[2] Adrianne K. Holmes,[1] Lorraine Backer,[1] Gene Burreson,[3] Marie C. Earley,[4] David Griffith,[5] Ronald Levine,[6] Wayne Litaker,[7] Joanne Mei,[4] Luke Naeher,[1] Larry Needham,[4] Edward Noga,[8] Mark Poli,[9] and Helen Schurz Rogers[1]

[1]Health Studies Branch, [2]Division of Environmental Hazards and Health Effects, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [3]Virginia Institute for Marine Sciences, College of William and Mary, Gloucester Point, Virginia, USA; [4]Division of Laboratory Services, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [5]Institute for Coastal and Marine Resources, East Carolina University, Greenville, North Carolina, USA; [6]University of North Carolina School of Public Health, Chapel Hill, North Carolina, USA; [7]Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill, North Carolina, USA; [8]North Carolina State University College of Veterinary Medicine, Raleigh, North Carolina, USA; [9]U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA

Since its identification in 1996, the marine dinoflagellate *Pfiesteria piscicida* Steidinger & Burkholder has been the focus of intense scientific inquiry in disciplines ranging from estuarine ecology to epidemiology and from molecular biology to public health. Despite these research efforts, the extent of human exposure and the degree of human illness directly associated with *Pfiesteria* is still in the process of being defined. Unfortunately, during this same time *Pfiesteria* has also stimulated media coverage that in some instances jumped ahead of the science to conclude that *Pfiesteria* presents a widespread threat to human health. Political and economic forces also came into play when the tourism and seafood industries were adversely impacted by rumors of toxin-laden water in estuaries along the east coast of the United States. Amid this climate of evolving science and public concern, *Pfiesteria* has emerged as a highly controversial public health issue. In October 2000 Centers for Disease Control and Prevention sponsored the National Conference on *Pfiesteria*: From Biology to Public Health to bring together *Pfiesteria* researchers from many disparate disciplines. The goal of this meeting was to describe the state of the science and identify directions for future research. In preparation for the conference an expert peer-review panel was commissioned to review the existing literature and identify research gaps; the summary of their review is published in this monograph. During the meeting primary *Pfiesteria* researchers presented previously unpublished results. The majority of those presentations are included as peer-reviewed articles in this monograph. The discussion portion of the conference focused upon researcher-identified research gaps. This article details the discussion segments of the conference and makes reference to the presentations as it describes emerging areas of *Pfiesteria* research. *Key words*: environmental health, estuarine ecology, harmful algae, *Pfiesteria*. — *Environ Health Perspect* 109(suppl 5):633–637 (2001).
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In 1996, reports of laboratory-based occupational exposure to an aerosolized marine toxin captivated both the scientific press (*1,2*) and the popular press (*3,4*). The following year, amid massive fish kills along the east coast of the United States, public health officials received reports of a variety of adverse health effects experienced by watermen who worked in estuaries where large numbers of fish were dying (*5–7*). During the same period a small number of recreational water users also reported signs and symptoms that they attributed to exposure to east coast estuarine waters (*8*). In many of these instances both the fish kills and the adverse human health effects were attributed to the presence of a newly identified dinoflagellate that had recently been given the scientific name *Pfiesteria piscicida* Steidinger & Burkholder (*8,9*).

By the summer of 1997, public concern was mounting, but little was known about the biology or ecology of *P. piscicida*, the toxicity of the organism, the possible routes of human exposure, or the potential adverse human health effects from environmental exposure to the organism. In an effort to better define the extent of the problem, public health officials from Centers for Disease Control and Prevention (CDC), eight state health agencies (Delaware, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, West Virginia) and the public health agency of the District of Columbia, the U.S. Food and Drug Administration, the National Institute of Environmental Health Sciences, and the U.S. Environmental Protection Agency convened to develop the public health response to possible human exposure to *P. piscicida* (*10*). The potential for adverse human health effects to occur on a larger scale than was currently being reported was a driving force behind this meeting, as well as the ensuing coordinated state and federal public health response to this emerging environmental threat.

On the basis of local conditions such as the degree of natural estuarine flushing, six states (Delaware, Florida, Maryland, North Carolina, South Carolina, Virginia) identified themselves as most likely to be affected by the presence of *P. piscicida* in their estuaries. CDC collaborated with these states to establish a uniform surveillance system for capturing all inquiries and symptom reports related to fish-kill events (*11*). Because no method existed to confirm exposure to *P. piscicida*, the collaborating public health agencies agreed to use exposure to estuarine waters as a surrogate for potential exposure to *P. piscicida*. From information about the initial self-reports of illness, and a preliminary investigation by CDC and state officials in Maryland, a series of exposure and symptom criteria were identified for possible estuary-associated syndrome (PEAS) (*5,12,13*). The state surveillance systems are supported by cooperative agreements with CDC and use toll-free numbers to facilitate reporting to state or local health departments. The surveillance system is designed to capture information about the human health effects potentially due to estuarine exposures and to tally public concern and the public health system burden associated with public inquiries about the unknown health implications of *Pfiesteria* exposure. Details of the multistate uniform surveillance system are reported in this monograph (*14*).

Concurrent with the initiation of surveillance, three states (Maryland, North Carolina, Virginia) embarked upon CDC-funded epidemiologic studies to assess adverse health effects among people who have the greatest likelihood of exposure to *P. piscicida*, such as watermen (*11*). Although the cohort studies

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Address correspondence to C. Rubin, CDC, 1600 Clifton Rd., NE, MS E-23, Atlanta, GA 30333 USA. Telephone: (404) 498-1373. Fax: (404) 498-1355. E-mail: crubin@cdc.gov

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are scheduled to continue through 2002, preliminary findings are reported in this monograph (*15,16*).

In October 2000 CDC sponsored the National Conference on *Pfiesteria*: From Biology to Public Health to bring together *Pfiesteria* researchers from many disparate disciplines actively engaged in *Pfiesteria*-related research. The goal of this meeting was to describe the state of the science and identify directions for future research. In preparation for the conference an expert peer-review panel was commissioned to review the existing literature and identify research gaps; the summary of their review is published in this monograph (*17*). During the meeting, primary *Pfiesteria* researchers presented previously unpublished results; the majority of those presentations are presented as peer-reviewed articles in this monograph (*18–34*). The discussion portion of the conference focused upon researcher-identified research gaps. This article summarizes those discussion segments and suggests directions for further *Pfiesteria* research.

Biology and Chemistry of *Pfiesteria* Species

Indiscriminate interchangeable use of the terms "*Pfiesteria piscicida*," "*Pfiesteria* species," and "*Pfiesteria*-like organisms" (PLOs) has been a source of confusion among both the scientific and lay communities concerned about *Pfiesteria*-related human health effects. The study of *Pfiesteria* and its biology relies on the accurate identification and differentiation of PLOs and provides a basic framework and foundation for all *Pfiesteria* researchers. Although initially *Pfiesteria* was identified using light microscopy, current technology for species identification uses scanning electron microscopy, plate tabulation (i.e., counting the number of plates that cover the organism), and recently developed molecular techniques (*26,33*). Unfortunately, these methods can be time consuming and costly, and they do not lend themselves well to field use. There is speculation that the reason no one has yet isolated *Pfiesteria* toxin from estuarine water samples is because it is a highly unstable molecule or family of molecules that quickly degrade, making identification and characterization of the toxin(s) particularly difficult (*35*). Because it has been difficult to differentiate *Pfiesteria* species quickly, a new functional species definition has been proposed that requires an organism to have a strong attraction to fresh fish tissues or excreta, toxic activity that is stimulated by the presence of live fish, and production of bioactive substances that can cause fish disease or death (*29*). However, the use of this ecological species definition instead of the more traditional molecular or morphologically based system currently in use needs further research and justification.

The life cycle of *Pfiesteria* species is complex and purports to include a number of amoeboid and chrysophyte cyst stages not seen in other marine dinoflagellates (*1,36*). Unfortunately, some laboratories engaged in *Pfiesteria* research report they have not been able to isolate or find any evidence for the amoeboid or chrysophyte cyst stage of the life cycle. Current research suggests that the transformation from amoebae to dinospore occurs only when the amoebae are exposed to fish (*29*), though the exact process has not been elucidated. This conclusion is complicated by the fact that fish have associated amoebae, and these amoebae are inevitably brought into the tanks with the fish. The overall absence of standardized culture protocols and mechanisms for interlaboratory validation of results represent crucial areas of future research.

Identifying and characterizing the putative *Pfiesteria* toxin and understanding what induces toxin production are central to understanding the impact the organism has on the environment and on human health. It is agreed in the research community that investigators should focus their efforts on identifying the suite of enzymes in *Pfiesteria* that triggers toxin production. Other organisms present in cultures could also cause the toxicity attributed to *Pfiesteria* (*29*). Although Glasgow et al. (*29*) reported that their *Pfiesteria* cultures were grown with antibiotics and antifungals, some researchers at the CDC-sponsored National Conference on *Pfiesteria* were skeptical that all estuarine bacteria were killed.

Future efforts should concentrate on the development of techniques to rapidly and accurately identify PLOs and *Pfiesteria* toxin in the field. The available molecular and morphological identification methods only confirm the presence of a particular species, not whether the species is capable of producing bioactive compounds that stress or compromise fish. Currently, only one laboratory facility is dedicated to producing and making available for analysis large quantities of culture water from tanks housing toxic *Pfiesteria*. Highly toxic cultures of *Pfiesteria* must be maintained on fish, which, when exposed to toxic *Pfiesteria*, rapidly die and release materials into the water. The resulting array of potentially bioactive compounds in the water further complicates the process of characterizing the toxin. Given the complexities of the identification process and the influence of potentially confounding factors, it is likely that significant progress in identifying the *Pfiesteria* toxin will be accomplished only by the collaboration and cooperation of many groups, all bringing to the research their own expertise and unique approaches to the problem.

Pathology of Diseased Estuarine Fish

Large numbers of dead or dying fish with characteristic punctate lesions (*37*) have been considered by both scientific and lay communities as indicating the presence of toxic *Pfiesteria* in estuarine waters. However, many contributing risk factors may lead to adverse fish health events in estuarine populations, and researchers must use caution when attributing a diseased state in fish to a single pathway. Diagnostic assessment to determine probable cause(s) of fish kills, lesions, or other adverse fish health events requires a multidisciplinary approach that includes epidemiology, water chemistry and pathology, and bacteriology and virology of affected fish. Although there has been much speculation about the actual role that *Pfiesteria* or PLOs play in the development of skin ulcers in fish, the gross ulcer is accepted as the end stage of a multifactorial event that needs to be examined from its earliest inception to gain a full understanding of the lesion development process (*38*).

Although the answers will come from careful laboratory research, public health officials must use current information to make recommendations about human exposure to estuarine waters. There are concerns that dismissing *Pfiesteria* as a primary cause of fish lesions may put people in harm's way, as people may perceive *Pfiesteria* to be a minimal risk to humans. All agree that the public needs to be educated about the possible significance of fish ulcers and disease and the potential relationship to human and environmental health. The presence of lesions or mortalities in a sensitive species of fish such as menhaden may serve as an important index in assessing the health of estuarine systems in general.

During discussions among researchers attending the CDC-sponsored conference, several gaps in current knowledge about *Pfiesteria* and PLOs were identified, including *a*) a lack of controlled laboratory studies to substantiate the current hypotheses regarding development of skin ulcer epidemics in estuarine fishery populations where *Pfiesteria*/PLOs are known or suspected to occur; *b*) a need for better understanding of the interaction between infectious and noninfectious risk factors that lead to skin ulcer development; *c*) a need for better understanding of the interaction among various infectious agents that lead to skin ulcer development; and *d*) the lack of biomarkers that identify the specific toxin(s) or other stressors that have caused damage to fish. Further research on diseased fish and control populations is needed before lesions and other manifestations of disease in fish can be attributed to the activity of *Pfiesteria*.

Estuarine Ecology and Environmental Monitoring

The science of *Pfiesteria* focuses not only on the organism itself but also on the environment in which it lives. Although researchers working with *Pfiesteria* are likely to have a basic understanding of broad issues such as general estuarine ecology, the specific ecology of *Pfiesteria* and environmental monitoring is more complex and includes topics such as eutrophication, nutrient enrichment, determination of causality in a fish kill, and state environmental monitoring efforts.

Monitoring the environment for the presence of *Pfiesteria* to gain an understanding about the circumstances under which the organism thrives is an important effort currently under way in six states (*21,22*). Because of the expense of field monitoring programs, clearly defined goals are needed so as not to burden human and financial resources. Standardized protocols must be implemented to allow state-by-state comparisons. The process of standardization takes time and is even further complicated because some research protocols, such as molecular diagnostics, are rapidly evolving. Hypothesis-driven monitoring offers the best chance of understanding the complex ecological relationships that control *Pfiesteria* abundance and that may eventually lead to predictive capability. Programs that monitor "everything everywhere" and then attempt to find significant correlations are probably not cost effective. Further, because a positive correlation cannot be interpreted as cause and effect, such programs may contribute little to scientific understanding in complex systems. Hypothesis-driven monitoring in conjunction with laboratory experiments to examine putative relationships is necessary to understand controlling mechanisms. Some states make use of citizen-based monitoring groups to provide better coverage of stations. It is generally agreed that citizens can play a valuable role, but potential problems exist with respect to data uniformity and quality. The lack of standardized training limits the usefulness of these groups. Another difficulty faced by volunteer monitoring groups is that the mere presence of *Pfiesteria* does not necessarily indicate a problem; *Pfiesteria* is often benign (*36,39*), unlike other harmful algal bloom organisms.

Directions for future research in the areas of estuarine and *Pfiesteria* ecology should focus upon furthering the understanding of the biochemistry and behavior of *Pfiesteria* and similar organisms in an ecological context and standardizing monitoring protocols. An important research objective is to identify the nature of the possible association between the presence of *Pfiesteria* and fish with lesions or other adverse health symptoms. Utilizing public interest in estuarine issues by providing appropriate training or outreach programs for volunteers anxious to collect environmental samples may increase the effectiveness of *Pfiesteria* research.

Molecular, Cell-Based, and Bioassay Methods for Detecting *Pfiesteria* Species and Their Suspected Toxins

A number of laboratory methods are currently in use or in development to detect and characterize *Pfiesteria* species and their suspected toxins. These methods include cell-based and molecular assays and a bioassay. Cell-based methods are used to detect the toxin and guide the fractionation processes (*23,36*), and molecular methods are used to detect the organism (*26*). Bioassay is used to confirm the presence of toxic *Pfiesteria* and to generate toxic cultures for fractionation and purification processes (*32*). Animal models are used for research into potential human health effects (*25*). The majority of research to detect the *Pfiesteria* organism and toxin incorporates any or all of these laboratory methods.

There is concern that quality assurance and quality control (QA/QC) principles are not being adequately applied to *Pfiesteria* research, particularly in the fish bioassay method. Because so little is known about the optimal conditions for growing and maintaining *Pfiesteria* cultures, it may be limiting at this time to set firmly the physical and chemical parameters. Laboratories involved in *Pfiesteria* research are encouraged to maintain good quality laboratory practices. At this stage of *Pfiesteria* research, it may be more beneficial for laboratories to report the conditions under which they successfully culture *Pfiesteria*. As more laboratories engage in QA/QC activities and apply them to culturing *Pfiesteria*, specific standardized ranges of physical and chemical properties can be determined. To improve the success rate of laboratories attempting to culture toxic *Pfiesteria*, it has been suggested that information relevant to the cultivation of these toxic cultures, as well as the cultures themselves, be shared among laboratories conducting *Pfiesteria*-related research (*32*). Fieldwide discussions or workshops to facilitate the exchange of knowledge and experience will provide researchers with the practical information needed to establish expertise.

In cell-based research, two receptors have been reported to be biologically responsive to the *Pfiesteria* toxin, the $P2X_7$ receptor and the *N*-methyl-D-aspartate (NMDA) neurotransmitter receptor (*23,40*). At this time there is insufficient data to allow comment on the proposed dual activity of the toxin. It is known, however, that other dinoflagellates have multiple sites for binding, and that the potency of some snake, spider, and scorpion venoms is due to their reaction with more than one receptor. The toxin-response mechanism will be better understood once a purified toxin is identified.

Studies on rats have provided models for evaluating the possible human health effects of exposure to *Pfiesteria* (*25*). Significant learning impairments have been identified in the rat model. However, no other nontoxic dinoflagellate species have been tested in this model, and no other pathology or follow-up experiments have been conducted on these rats. Though rats in this study have not yet been injected with a $P2X_7$ agonist, they have been injected with an antagonist for the second receptor, NMDA. Rats injected with the NMDA antagonist became neurologically deficient, though the deficiencies were different from those caused by direct injection of *Pfiesteria*.

Future research will undoubtedly focus on the isolation, purification, and characterization of the *Pfiesteria* toxin(s). This will improve existing methods and aid researchers in developing new methods to detect the presence of the toxin in laboratory and field samples. Methods in development to identify the *Pfiesteria* organism include antibody probes, lectin probes, electrochemical methods, and fluorescent fragment detection. There is also work under way to differentiate among life cycles by stage-specific reverse transcriptase-polymerase chain reaction. In studies to determine potential human health effects, future research will involve the application of extracts of *Pfiesteria* culture to specific areas in rat brains to study behavior and pathology.

Communication and Public Relations Issues Related to *Pfiesteria*

The possible human health impact of a newly emerged toxin in east coast waterways caught immediate media attention and created a public perception that waters were not safe for recreation and consumption of fish could be dangerous. State-based communication specialists were faced with the challenge of protecting public health by communicating an appropriate level of concern while also representing tourism and fishing in an accurate perspective. Many states initiated hotlines and used state- and federally sponsored web sites to disseminate information quickly (*19*). However, anecdotal information that engenders fear continues to be a concern for communication and public relations specialists working on issues relating to *Pfiesteria*.

Discussions during the 2000 conference in Atlanta identified several points that researchers felt should be considered in order to improve the effectiveness of *Pfiesteria*-related communications efforts. State officials stressed the importance of presenting anecdotal or limited case reports in a broader context. For example, millions of people use the waters of the Chesapeake Bay and the Pamlico Sound annually without experiencing any sickness, but this point is frequently lost when a single swimmer reports adverse effects and the media implicate *Pfiesteria*. There was general consensus that hot lines, websites, and public meetings are important ways to respond to the often contradictory and problematic information available about *Pfiesteria* and to raise consciousness about other pressing environmental matters (*19*). In addition, the conference participants voiced that researchers and public health officials should be careful to avoid assigning blame or to overstate or understate the health threat of *Pfiesteria*. Past carelessness has resulted in an overly alarmed public and a farming community whose members believe they have been mistakenly maligned. It is important to provide accurate and up-to-date information about ongoing research to raise public awareness without causing alarm. To this end, public health agencies should refer to and use peer-reviewed literature in developing *Pfiesteria* fact sheets.

On a positive note, the *Pfiesteria* issue has resulted in a great deal of multidisciplinary exchange of information and ideas and has produced an active multi-interest group conversation about environmental issues. This multidisciplinary involvement can only strengthen *Pfiesteria*-related communications and benefit the public.

P. piscicida and Human Health

It is debatable whether it is appropriate at this time to use traditional epidemiological tools (e.g., surveillance and cohort studies of highly exposed people) to study the potential human health effects of *Pfiesteria*. When the original state-based studies were defined, researchers were confident that identification of a biomarker of human exposure to *Pfiesteria* toxin was imminent. Development of such a biomarker has proved to be much more complex than anticipated and has severely impaired the usefulness of epidemiological studies. The findings of ongoing cohort studies will have to be interpreted with great caution, especially since there has not been a large-scale *Pfiesteria*-related fish kill since 1997. However, cohort study investigators note that the ongoing studies carefully address low-dose chronic exposure (*15*) and strive to identify subtle neurological effects. Biological samples are being banked for analysis when a serological test is developed.

Human health effects could be most definitively assigned if researchers conducted a controlled volunteer-exposure study. However, such studies cannot yet be conducted because in the absence of the toxin, the long-term effects that may be associated with *Pfiesteria* exposure are as yet undefined.

Recent research has suggested that visual contrast sensitivity (VCS) testing may be an informative tool for diagnosing PEAS (*16,41*). PEAS is the syndrome associated with the collection of signs and symptoms exhibited by persons who report exposure to estuarine water or laboratory or aquaculture work involving estuarine water within 2 weeks prior to symptom onset (*14,42*). VCS testing is controversial because abnormal findings are not toxin-specific and abnormal findings can be interpreted only cautiously unless accompanied by other fairly extensive visual tests. At this point, a battery of visual performance testing is not compatible with the cohort-testing format.

The need for a biomarker of human exposure to *Pfiesteria* toxin remains the single critical barrier to defining human health outcomes related to exposure to *Pfiesteria* toxin. To better interpret clinical findings, especially subtle findings that may more accurately be attributed to agents other than *Pfiesteria*, it is critical that a field-based test to assess *Pfiesteria* toxin in water samples and a test to identify a marker of exposure in human biological samples be developed.

Conclusion

The future direction of *Pfiesteria* research can and should be guided by discussion among researchers who are actively engaged in one of the varied components of *Pfiesteria*-related research. When these researchers met in Atlanta in the fall of 2000, they identified two research areas of primary importance in filling existing knowledge gaps and advancing the science so that we can address more definitively the question of the threat that *Pfiesteria* poses to human health. Conference attendees agreed that identification of the *Pfiesteria* toxin and the development of a test for biomarkers of the toxin in humans were the most crucial areas for *Pfiesteria* research. It was further recognized that achieving these goals would require collaboration and sharing of information among individuals currently involved in various aspects of *Pfiesteria* research. It is only with a multidisciplinary and collaborative approach that the environmental and public health significance of *Pfiesteria* will be fully understood.

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