

Human Health Effects and *Pfiesteria* Exposure: A Synthesis of Available Clinical Data

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An association between human illness and exposure to *Pfiesteria* was first observed among laboratory personnel working with the microorganism. In 1997, in the setting of *Pfiesteria* activity on the Pocomoke River in Maryland, difficulties with learning and memory were epidemiologically associated with high-level exposure to waterways in which the organism was known to be present. In the Maryland studies, neurocognitive function of affected persons returned to within normal ranges within a period of 3–6 months. Persons with the most severe neurocognitive deficits were significantly more likely to have skin lesions characterized on biopsy by evidence of a toxic/allergic inflammatory reaction. Acute high-level exposures to waterways where *Pfiesteria* has been identified have been linked with eye and respiratory irritation, headache, and gastrointestinal complaints. Recent data, collected using molecular techniques, suggest that the organism is present in multiple locations in the Chesapeake Bay environment; available data are insufficient to comment on the possible cumulative health impact of chronic low-level environmental exposure to *Pfiesteria*. **Key words:** dinoflagellate, memory, *Pfiesteria*. — *Environ Health Perspect* 109(suppl 5):787–790 (2001). <http://ehpnet1.niehs.nih.gov/docs/2001/suppl-5/787-790morris/abstract.html>

Dinoflagellates in the genus *Pfiesteria* were first identified in the early 1990s by JoAnn Burkholder and colleagues at North Carolina State University, Raleigh, North Carolina, in association with fish kills in the Pamlico and Neuse estuaries in North Carolina (1). These studies further suggested that exposure to toxic forms of the organism contributed to the appearance of characteristic “punched out” skin lesions in fish (1,2) [an assumption that has been the source of some recent controversy (3)].

Shortly after the initial description of *Pfiesteria*, questions were raised about the human health impact of contact with the organism and its toxin(s). Laboratory personnel working with *Pfiesteria* became ill (4,5), and there were subsequent reports of illness associated with environmental exposure to waterways where the dinoflagellate was present (4,6). Although questions remain about the linkage between *Pfiesteria* and the observed clinical syndromes, there are suggestions that chronic or recurrent high-level exposure to *Pfiesteria* and its toxin (i.e., exposure to *Pfiesteria* at a time when there is evidence of toxin production, as manifested by fish kills and/or a high frequency of fish with skin lesions) produces a distinctive clinical syndrome characterized by difficulties in learning and memory. More transient manifestations (confusion, flulike symptoms) have been reported in association with a single intense exposure (7).

This article provides a synthesis of these and other available clinical data, with a focus on studies conducted by investigators who were part of the joint University of Maryland/Johns Hopkins University research group on *Pfiesteria*. Given the

recent recognition of this clinical entity, its apparent rarity, and the lack of a definitive diagnostic test for exposure, data have, of necessity, been drawn from multiple sources, including peer-reviewed articles, conference presentations, abstracts, and newspaper reports.

Clinical Syndromes Associated with *Pfiesteria*

Chronic/Recurrent High-Level Exposure

North Carolina: laboratory exposure. The first indication that toxins produced by *Pfiesteria* could cause human illness came when investigators working with the organism in Burkholder’s laboratory in North Carolina began to experience problems with respiratory and eye irritation, skin rashes, gastroenteritis (stomach cramps, nausea, vomiting) and of particular concern, cognition and personality changes. These data were initially reported by Glasgow et al. (4); a more detailed report of neurologic and neurocognitive findings from one of the most severely affected individuals has recently been presented (5). Although most symptoms appear to have resolved, concerns remain among affected investigators about persistent effects (including persistent neurocognitive deficits) 7–8 years after the acute incident.

The exposures in these cases appear to have been both high level (exposure to water from tanks containing *Pfiesteria* showing evidence of toxin production) and recurrent (occurring repeatedly during a period of 2–3 months). It was postulated that exposure was due both to direct water contact and inhalation of aerosols from toxic tanks. The level of exposure for some investigators may have been increased by a construction error that

resulted in venting of air from the room where toxic cultures were being prepared into an office area.

Maryland: Pocomoke River. In the fall of 1996, Maryland commercial fishermen (watermen) working along the Pocomoke and other small rivers emptying into Tangier Sound on the eastern shore of the Chesapeake Bay (Figure 1) began to note increasing problems with fatigue, headaches, respiratory irritation, and memory disturbances. Persons who were most severely affected worked primarily on the Pocomoke River, often with ≥ 6 hr of water exposure per day, 6 days/week, extending over several months. Some were bank trappers who collected fish from traps set up near the bank of the river; these persons reported that, at times, 70–80% of the fish collected from the traps were moribund or had “punched out” skin lesions.

Using light and scanning electron microscopy, Burkholder and colleagues from North Carolina State University identified *Pfiesteria* [*Pfiesteria piscicida* Steidinger & Burkholder and other *Pfiesteria*-like species (1)] in water samples collected from the Pocomoke River during the summer of 1997. After fish kills occurred on the river in August 1997, the Maryland Department of Health and Mental Hygiene asked investigators at the University of Maryland and Johns Hopkins to evaluate health complaints among watermen. Ultimately, 24 case persons were evaluated. All had direct contact with the Pocomoke River and/or other estuarine waters of the Chesapeake Bay during periods of fish kills or at times when a high

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Much of the data summarized in this article were developed by our Maryland *Pfiesteria* team. Key participants have included R. Venezia and A. Chapin and their staff at the Maryland Department of Health and Mental Hygiene; D. Oldach, L. Gratten, R. McCarter, and L. Wilson and colleagues at the University of Maryland School of Medicine; and T. Perl at Johns Hopkins School of Medicine.

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percentage of fish had skin lesions (6). This included seven of the eight watermen who regularly worked on the Pocomoke River, state employees having contact with the river as a result of official duties, and persons with recreational water contact. Exposed persons were divided by degree of exposure [severe, contact with affected waterways 6–8 hr/day on an almost daily basis; moderate, 8–20 hr/week assessing fish or collecting water samples from affected waterways (including, in some cases, minimal water contact, but frequent exposures to water aerosols while scooping up dead fish); low, little or no direct skin contact with water but had ridden in boats on the Pocomoke during fish kill events]. Controls were selected for each case person, matching on age, sex, occupation, and educational level. Watermen controls were selected from among watermen working on the ocean side of the Delmarva peninsula, where lesioned fish had not been seen.

Physical examination and clinical laboratory studies of exposed persons were generally unremarkable (6). Persons with severe levels of exposure were significantly more likely than controls to complain of neuropsychological problems (confusion, episodes of disorientation, new or increased forgetfulness, or difficulties concentrating; 82 vs 13%, $p < 0.01$), headaches (82 vs 13%, $p < 0.01$), and skin lesions (73 vs 13%, $p < 0.05$). Skin lesions were reported as erythematous, edematous papules on the trunk or extremities (8) and generally resolved within a few days to a week. On careful dermatologic evaluation, lesions appeared to have a variety of etiologies. However, on skin biopsy there was a subset of lesions (from 4 of 12 patients) that had similar histopathologic findings, suggestive of an inflammatory, toxic, or allergic process (8,9); these same four patients had the most severe neurocognitive changes, as described below ($p < 0.01$) (9).

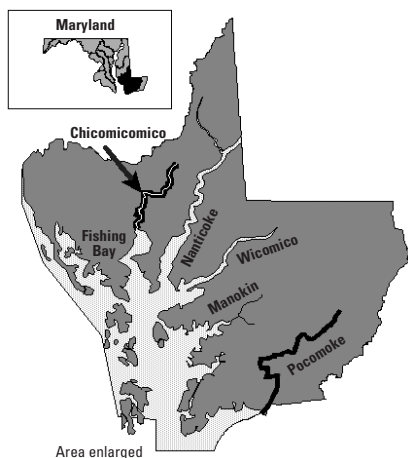


Figure 1. Chesapeake Bay Region.

Significant associations were found between the degree of exposure to *Pfiesteria*-affected Maryland waterways and deficiencies in learning, memory, and higher-order cognitive function, as measured by formal neurocognitive testing [see Grattan et al. (6) for a complete description of tests and analysis of results]. Results were most striking on the Rey Auditory Verbal Learning and Memory Test (Rey AVLT), which measures the ability of persons to remember word lists at successive time points. As shown in Table 1, there was a significant difference between the four exposure groups with respect to performance on trial 5 of the Rey AVLT, with the degree of deficit corresponding to degree of exposure. The primary deficit appeared to be in the ability of patients to place new observations into memory (i.e., to learn). In contrast, material that had already been learned could generally be recalled without difficulty.

Patients in this study tended to be young, male, self-employed, and otherwise healthy; on tests of mood state, most ranked as vigorous. Findings were not consistent with a hysterical reaction (10), and persons with any suggestion of malingering or symptom exaggeration or who had any other possible explanation for the observed deficits were excluded from analysis. When patients (and controls) were formally retested 3 and 6 months after cessation of exposure, patient test scores increased significantly, returning in all instances to a normal range (6). Although deficits in most instances appear to have resolved completely, some patients continue to feel that they are not “back to normal.”

Virginia: Pocomoke River. The Pocomoke River lies on the border between Maryland and Virginia on the Delmarva peninsula. Using test batteries similar to those employed in Maryland, investigators working with the

Virginia health department tested four persons. Two of the four persons (both of whom had “extensive, daily exposure” to the Pocomoke River during the summer of 1997) were reported to show evidence of mild to moderate short-term memory loss and problems with focusing their attention. Both also had abnormalities on magnetic resonance imaging scans; researchers felt these abnormalities did not explain the observed deficits (11).

Possible Acute High-Level Exposure

Maryland: Chicamacomico River. Six of seven state employees sent to investigate a fish-distress/kill event on the Chicamacomico River (a river close to the Pocomoke that also empties into Tangier Sound on the Maryland Eastern Shore) reported acute respiratory and eye irritation, headache, and/or sore throat within 4 hr of exposure to the river (7). None of these persons were included in the Pocomoke River study described above. Three of the affected persons had direct water contact, whereas for the other four, contact was primarily from aerosols coming off the river. The weather was described as warm (80°F), humid (100%), and windless, with workers reporting a heavy mist rising off the slow-moving water. During the event, four developed burning eyes or nares, and six developed a headache or sore throat. Six developed crampy abdominal pain, nausea, or diarrhea within 4 hr of their exposure. However, when formal neuropsychological testing was done within 3 weeks of the event (and again 3 months later) no consistent pattern of deficit was seen. Symptoms generally resolved within a week. Skin lesions were not reported.

The fish-distress/kill event was localized to a length of the river of approximately 600 yards; at its height, an estimated 75,000–450,000 Atlantic menhaden were distressed, with virtually 100% of fish sampled showing skin lesions. A water sample collected at the time had a dissolved oxygen value of 7.7 mg/L and a pH of 7.1. *P. piscicida* was present in water samples collected during the acute event, based on scanning electron microscopic studies conducted by K. Steidinger; samples were also toxic in fish assays conducted by J. Burkholder (7).

North Carolina/Maryland: case reports.

There are case reports from both North Carolina (4) and Maryland (12,13) suggesting an association between exposure to waterways known to have *Pfiesteria* activity and the appearance of one or more of a combination of symptoms, including skin lesions, headache, gastrointestinal symptoms, eye and respiratory irritation, and mental confusion. Although documentation is not always optimal, symptoms generally appear to have resolved within a week or so of the acute exposure.

Table 1. Means, standard deviations, and ANOVA for Scores on two experimental neuropsychological measures for case persons and controls, Pocomoke River study in Maryland.^a

	Number	Mean (SD) Rey AVLT trial 5 T score ^b	Mean (SD) Stroop Interference T score ^c
Exposure			
None	19	54.21 (7.32)	51.58 (7.99)
Mild	3	35.33 (14.74)	39.00 (2.00)
Moderate	7	38.57 (13.15)	48.83 (11.36)
Severe	9	19.56 (16.69)	39.78 (10.87)
Results			
ANOVA		$p < 0.0001$	$p < 0.0001$
Linear trend		$p = 0.011$	$p = 0.006$

ANOVA, analysis of variance.

^aData from Grattan et al. (6). ^bRey AVLT measures the capacity for new verbal learning and memory for complex verbal information. ^cStroop interference test, from Stroop Color-Word Test; assesses resistance to interference and selective attention.

Chronic Low-Level Exposure

There are now increasing data that support the concept that *P. piscicida* and other *Pfiesteria* species are relatively widely distributed in the estuarine environment. For example, in studies conducted in Maryland by Oldach and colleagues, employing a real-time PCR assay for *P. piscicida*, the organism was detected at multiple estuarine sites in Chesapeake Bay (14). Detection was most common in late summer and at sites with higher nutrient and phytoplankton biomass. The potential for toxicity among these isolates remains controversial (2). Nonetheless, the presence of the organism at multiple sites raises questions about possible human health effects of low-level exposure [exposure to the organism in a setting in which there is no or minimal toxin production (as reflected by the lack of major fish kills or minimal numbers of lesioned fish) and/or very limited exposure to waterways in which there is evidence of toxic *Pfiesteria* activity].

Griffin and colleagues, in a 1996 study in North Carolina, compared reported symptoms among two groups of crabbers (those working in and those working outside of waters where fish kills in which *Pfiesteria* has been implicated as a cause are known to have occurred) and a third group of nonfishing individuals (15). For most conditions, subjects in all three groups reported comparable levels of injury and illness. Fatigue was more commonly reported by crabbers working in fish-kill areas than in areas without fish kills (9.4 vs 3.5%, $p = 0.05$); however, this was felt to be attributable to the participants' perceptions of "overwork" or the presence of other underlying medical conditions rather than water quality. The ratio of well to ill crabbers was slightly lower for crabbers working in the two areas known to have experienced fish kills. These studies were based on self-reported symptoms and did not include formal neuropsychological testing.

Formal testing was included in a subsequent North Carolina study looking at smaller groups of watermen working in waterways where diseased or stressed fish were reported from June to September 1997, and where *Pfiesteria* has been reported in the past ($n = 22$), and controls from unaffected waterways ($n = 21$). Testing in this study was performed 3 months after the last documented fish kill in affected waters. No differences were found among groups, with one exception: Persons with exposures to affected waterways had significant deficiencies in visual contrast sensitivity, an indicator of impairment in human visual-system function. Findings are similar to those reported among organic solvent-exposed workers; however, cases (exposed persons) and controls in this study did not differ in their history of exposure to

such compounds (16,17). These findings have led Shoemaker and Hudnell to propose adoption of visual contrast sensitivity (as measured by the Functional Acuity Contrast Test) as the key diagnostic test for *Pfiesteria* syndrome (13).

Summary and Discussion

The strongest data for a link between *Pfiesteria* and its toxins and human illness are those associated with the laboratory exposures in the Burkholder laboratory. The Pocomoke River studies (and the concurrent, albeit much more limited, Virginia studies) identified a group of patients with a distinctive clinical syndrome, characterized by difficulties with learning and memory, in an environmental setting in which *Pfiesteria* was known to be present. The data from these latter studies are not sufficient to say that *Pfiesteria* or *Pfiesteria* toxin were the immediate cause of the observed illness, and there remain questions about the possible contribution to illness of other toxic algal species that may have been present. Nonetheless, it is a plausible hypothesis, given the similarities in neurocognitive symptoms seen among laboratory- and environmentally exposed persons, and the work by Levin demonstrating learning problems in rats exposed to toxic material from *Pfiesteria* (18–20). These observations further suggest that development of learning and memory problems (if due to *Pfiesteria*) requires recurrent, presumably high-level exposure. Symptoms appear to resolve within 3–6 months of exposure (6), although concerns remain about long-term sequelae in severely affected persons. At a much more anecdotal level, there are suggestions that an episode of intense exposure to waterways in which toxic *Pfiesteria* is present can result in an acute syndrome characterized by eye and respiratory irritation, headache, fatigue, and/or gastrointestinal symptoms (7).

As noted above, some investigators have proposed reliance on visual contrast sensitivity as the basis for diagnosing *Pfiesteria* syndrome (13). Data supporting use of the test in this setting are weak. Visual contrast sensitivity is affected by underlying eye disorders, including corneal and lens disorders (i.e., the effect on the contrast sensitivity function is optical), as well as retinal and optic nerve disorders. As the individuals most likely to experience possible *Pfiesteria* toxin exposure are those individuals spending significant amounts of time on the water, there is a heightened probability of ultraviolet exposure-associated eye disease, such as lenticular opacity and age-related macular degeneration. Thus, a positive screening test for impaired contrast sensitivity may simply reflect the environmental/occupational context for the tested individual. There is clearly a need for further research in this area.

The physiologic mechanisms underlying the observed memory and learning deficits remain obscure. Recent work by investigators at the University of Maryland has raised the possibility that bioactive material from *Pfiesteria* acts as an *N*-methyl-D-aspartate (NMDA) neurotransmitter receptor antagonist (21). The NMDA receptor plays a critical role in neural plasticity and learning (22,23); the observed learning and memory deficits in patients could plausibly be attributed to a toxin having this type of biologic activity. In terms of route of exposure, it is possible that the toxin/toxic material is transmitted by aerosols. By analogy, aerosolization of toxin/toxic material has been implicated as the cause of respiratory irritation reported in association with exposure to *Gymnodinium breve* red tides on the east coast of Florida (24). Skin contact with water and/or affected fish may also be a risk factor. In contrast, consumption of fish or shellfish has not been implicated as a cause of the described illnesses (25). Maryland and other mid-Atlantic states have developed policies for closure of waterways when the organism appears to be present and active, as manifested by fish kills or signs of toxicity in fish. Until there is a better understanding of *Pfiesteria* toxins and their mode of action, it would appear prudent for persons who must come in contact with such waterways, particularly in the midst of an active *Pfiesteria*-related fish kill, to wear protective clothing and respiratory protective gear.

Intense surveillance conducted since 1997 in Maryland and other mid-Atlantic states suggests that the clinical syndrome described in association with intense, high-level exposures to toxic *Pfiesteria*, as seen in the Burkholder laboratory and along the Pocomoke River, is rare. The North Carolina crabber study (15) further suggests that routine occupational (and recreational) exposure to waterways where *Pfiesteria* is known to be present has minimal health effects, at least at a subjective level. From a public health standpoint (and in light of the previously noted relatively wide distribution of *Pfiesteria* in the environment) the key question would appear to be whether there is any cumulative effect resulting from chronic, low-level exposure to the organism and its toxin in the environment. The cohort studies currently under way in Maryland, Virginia, and North Carolina, sponsored by state health departments and the Centers for Disease Control and Prevention, may help to address this critical question.

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