

Cohort Studies of Health Effects among People Exposed to Estuarine Waters: North Carolina, Virginia, and Maryland

Christine L. Moe,¹ Elizabeth Turf,² David Oldach,³ Paula Bell,¹ Steve Hutton,¹ David Savitz,¹ Deborah Koltai,⁴ Megan Turf,² Lily Ingsrisawang,² Robert Hart,⁵ J.D. Ball,⁶ Michael Stutts,⁶ Robert McCarter,⁷ Leslie Wilson,⁷ Dirk Haselow,⁷ Lynn Grattan,⁸ J. Glenn Morris,⁷ and David J. Weber¹

¹Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; ²Virginia Commonwealth University, Survey and Evaluation Research Laboratory, Richmond, Virginia, USA; ³Department of Medicine and Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland, USA; ⁴Departments of Psychiatry and Medicine, Duke University Medical Center, Durham, North Carolina, USA; ⁵Department of Psychiatry, Virginia Commonwealth University, Richmond, Virginia, USA; ⁶Neuropsychology Center, Eastern Virginia Medical School, Norfolk, Virginia, USA; ⁷Departments of Epidemiology and ⁸Neurology, University of Maryland School of Medicine, Baltimore, Maryland, USA

A variety of human symptoms have been associated with exposure to the dinoflagellate *Pfiesteria* and have been grouped together into a syndrome termed "possible estuary-associated syndrome." Prospective cohort studies of health effects associated with exposure to estuarine waters that may contain *Pfiesteria* spp. and related organisms are in progress in North Carolina, Virginia, and Maryland. The three studies recruited cohorts of 118–238 subjects who work or engaged in recreation in estuary waters. Baseline health and neuropsychological evaluations are conducted, and study subjects are followed prospectively for 2–5 years with periodic assessments of health and performance on a battery of neuropsychological tests. Health symptoms and estuary water exposure are recorded by telephone interviews or diaries every 1–2 weeks. Water quality information, including measurements of *Pfiesteria* spp., is collected in the areas where the subjects are working. Because it is not possible to measure individual exposure to *Pfiesteria* or a toxin produced by this organism, these studies examine surrogate exposure measures (e.g., time spent in estuary waters, in a fish kill area, or in waters where *Pfiesteria* DNA was detected by molecular amplification). Preliminary analyses of the first 2 years (1998–2000) of data indicate that none of the three ongoing cohorts have detected adverse health effects. However, there have not been any reported fish kills associated with *Pfiesteria* since the studies began, so it is possible that none of the study subjects have been exposed to toxin-producing *Pfiesteria* spp. **Key words:** dinoflagellates, estuary-associated syndrome, fishermen, neurotoxin, occupational health, *Pfiesteria*. — *Environ Health Perspect* 109(suppl 5):781–786 (2001).

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A variety of human symptoms and signs have been reported to be associated with exposure to the dinoflagellate *Pfiesteria*, especially neurological dysfunction and skin lesions (1–3). On the basis of preliminary studies of affected laboratory workers and fishermen, the constellation of clinical findings associated with *Pfiesteria* exposure has been grouped into a syndrome termed "possible estuary-associated syndrome" (PEAS) for research purposes (4,5). In the present article, we describe the methods and preliminary results of three prospective cohort studies undertaken to elucidate the risks and possible clinical disease associated with exposure to *Pfiesteria* spp. being conducted in North Carolina, Maryland, and Virginia.

Background

Pfiesteria spp., a genus of dinoflagellates, were first described in laboratory studies of fish mortality (6) and in association with major fish kills in North Carolina (7). Since that time, *Pfiesteria* spp. have been detected in waters with fish kills from the mid-Atlantic to the gulf coast. Laboratory studies indicate that these organisms have a complex life cycle that includes a toxic stage (1). Ulcerative

lesions, narcosis, erratic behavior, and death have been observed in several fish species exposed to active cultures of *Pfiesteria* spp. and *Pfiesteria*-like organisms (8).

Public concern about possible human health hazards posed by *Pfiesteria* spp. began in 1995 when adverse health effects were reported among investigators working with this organism in the laboratory (9). Glasgow et al. described three laboratory workers with exposure to *Pfiesteria* spp. cultures via direct contact with hands and arms and potential inhalation of aerosols from open aquaria. The exposed persons experienced various combinations of symptoms that included numbness and tingling in hands and feet, skin lesions, respiratory and eye irritation, headaches, abdominal cramps, difficulties with mental concentration and memory, and personality changes. Most prominent symptoms subsided after cessation of exposure, although resolution for some symptoms took several months.

Several epidemiological studies have attempted to examine possible human health risks associated with exposure to fish kills or estuarine waters. Griffith (10) conducted a cross-sectional study in North Carolina of 23

crabbers in the Pamlico Estuary (an area affected by fish kills associated with *Pfiesteria* spp.), 115 crabbers in the Albemarle area, and 125 nonfishing community controls. All three groups reported similar levels of illness and injury, except that both groups of crabbers reported higher prevalence of skin problems than the community controls. Exposure to water or locations with fish kills did not appear to be associated with increased risk of illness. In August 1997, a fish kill occurred on the Pocomoke River in Maryland that triggered a study of 24 individuals with varying degrees of exposure to the Pocomoke waters and other estuaries in the Chesapeake Bay and also of eight unexposed watermen (11). Subjects were asked about water exposure and health symptoms, examined by a medical team, and tested with a neuropsychological screening battery. Individuals who reported high exposure (6–8 hr/day in affected waterways, with extensive skin contact and exposure to aerosolized spray) were significantly more likely than occupationally matched controls to complain of neuropsychological symptoms, headache, skin lesions, or a burning sensation of skin on contact with water. Nineteen study subjects exposed to affected waters had significantly reduced scores compared to 19 nonexposed subjects (matched on age, gender, education, occupation) on three neuropsychological tests that indicated difficulty with learning and higher cognitive function. The investigators reported a dose–response effect in that study subjects who reported the greatest exposure to the affected waters had the lowest scores on selected neuropsychological tests. No consistent abnormalities were

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Address correspondence to C.L. Moe, Dept. of International Health, Rollins School of Public Health of Emory University, 1518 Clifton Rd., NE, Atlanta, GA 30322 USA. Telephone: (404) 727-9257. Fax: (404) 727-4590. E-mail: cmoe@sph.emory.edu

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detected in the physical examinations or laboratory assessments of the study subjects. As with the reported cases among laboratory workers, the major neurocognitive problems appeared to be transitory, and the test scores for all study subjects returned to within normal ranges by 3–6 months after cessation of exposure. However, preexposure cognitive test performances for these individuals are not known, and some study subjects complained of persistent symptoms for longer periods. This study is the first systematic investigation of human health effects after exposure to a fish kill associated with *Pfiesteria* spp. However, the study was limited by several factors: *a*) the number of subjects was small and subjects were largely self-selected, *b*) exposure status was self-reported and may have been affected by recall bias, and *c*) the medical team was not blinded to the exposure status of the subjects during the medical and neuropsychological evaluations.

In a November 1997 study, 17 gill net and crab pot fishermen and four state employees who worked in two estuaries (Neuse and Pamlico) in North Carolina with a recent history of fish kills or fish with lesions and from which *Pfiesteria* spp. were detected were examined and compared to a group of 21 watermen who had little or no exposure to the affected waters (primarily ocean fishermen) (12). The occupationally exposed group was recruited from a roster of licensed commercial fishermen provided by the North Carolina Division of Marine Fisheries. Both the exposed and nonexposed groups were recruited by telephone and matched for age and education. All study subjects had a comprehensive medical evaluation with dermatological and neurological assessments, vision testing, and neuropsychological evaluations, and the investigators were blinded to the exposure status of the subject at the time of testing. No significant differences in health or neurocognitive performance were observed between the groups working in the affected waters compared to the unexposed group. However, the estuary fishermen group had approximately a 30% reduction in visual contrast sensitivity (VCS) compared to the ocean fishermen (13). Multiple linear regression analyses indicated that age, smoking, and time spent on any water accounted for some of the variance in VCS at a middle spatial frequency, and there was significant interaction between exposure group and age. However, exposure to estuary water was the most significant predictor of decreased VCS, and this group did not show the normal trend of decreasing VCS with age as seen in ocean fishermen, suggesting that another factor was affecting VCS in estuary fishermen. Again, this study included a small number of subjects. Exposure status was self-reported and may reflect recall bias. In

addition, this study was conducted more than 3 months after the last documented fish kill associated with *Pfiesteria* and thus could only examine persistent health effects that may have been related to this exposure.

Participants at a workshop sponsored by the Centers for Disease Control and Prevention (CDC) in 1997 proposed the term “possible estuary-associated syndrome” (PEAS) to describe the adverse consequences of exposure to *Pfiesteria* spp. and related organisms (4). To identify potentially affected persons and determine the extent of the public health risk posed by environmental exposure to *Pfiesteria* spp., public health officials initiated a multistate surveillance program in 1998. The PEAS symptom and exposure criteria proposed by the CDC-sponsored multistate workshops were first summarized and published in 1997 (4) and modified in 1999 (9). The key criteria included in the definition were *a*) exposure to estuarine water with a fish kill or fish with lesions consistent with *Pfiesteria* spp. or *Pfiesteria*-like organisms, *b*) symptoms of memory loss and confusion, *c*) three or more other symptoms from a list of clinical features that have been reported in previous incidents of *Pfiesteria* exposure, *d*) symptoms developing within 2 weeks of exposure to estuarine water and persisting for 2 weeks or longer, and *e*) the inability of the healthcare provider to identify another cause for the symptoms.

In 1997, Congress directed the CDC to conduct studies on possible human health effects associated with exposure to *Pfiesteria* spp. The CDC coordinated multistate meetings in late 1997 and early 1998 with representatives of state health departments, environmental quality departments, and several academic institutions to design epidemiological studies of human health effects associated with exposure to estuarine waters that may have *Pfiesteria* spp. In March 1998, the CDC funded three prospective cohort studies in North Carolina, Maryland, and Virginia. The primary research objective was to determine if exposure to estuarine waters, especially estuarine waters in which *Pfiesteria* spp. are known to be present, is associated with adverse health effects. These three studies are currently in progress. The overall design and preliminary results for all three studies are presented in this article.

Materials and Methods

Overall Study Design

Because of the array of symptoms associated with PEAS, a prospective cohort study design was chosen to allow the evaluation of multiple health outcomes as well as assess the temporal relationship between exposure and disease. In addition, information could be

collected on individual risk factors, such as alcohol consumption and solvent exposure, and the effects of these factors controlled in the analyses. Cohorts of commercial fishermen and others who spend a substantial amount of time working or recreating on estuary waters were recruited. Baseline evaluations at the time of recruitment included a medical history and general health assessment, medical examination, neuropsychological assessment using a battery of standardized tests, evaluation of vision, and exposure assessment. Follow-up evaluations, similar to the baseline evaluation, are performed twice per year for 2–5 years. Health effects and exposure are monitored by telephone interviews every 1–2 weeks or by use of a logbook. Study protocols and data collection tools were reviewed and approved by the Committee for the Protection of Human Subjects at each institution involved in the study.

Cohort Recruitment and Characteristics

The cohorts in the three states were recruited by a variety of approaches. Letters explaining the study and inviting participation were mailed to registered commercial fishermen and followed by telephone contact. Presentations were made at fishing trade shows, watermen association meetings, and fishing community events such as the annual Blessing of the Fleet. Study brochures were distributed to fish markets and warehouses. Radio and television announcements and articles in local newspapers and watermen association newsletters were also used to publicize the study. Maryland and Virginia started recruiting their cohorts in the spring of 1998. Virginia enrolled subjects through the spring of 1999 and Maryland through June 2000. The North Carolina study started 1 year later than the studies in Maryland and Virginia and had a 1-year enrollment from April 1999 to May 2000.

The target study population was individuals who spent a substantial amount of time out on the water in selected coastal environments in Maryland, Virginia, and North Carolina (Figure 1). Also included were some individuals who were likely to have little or no exposure to estuary waters, such as ocean fishermen or members of the community who lived in coastal areas but did not work on the water. There were some differences in the cohort inclusion criteria for the three states. Study subjects had to be between 18 and 70 years of age (18–65 years of age in North Carolina). Subjects in North Carolina had to work on study estuaries (Albemarle, Pamlico, Neuse) or the ocean for ≥ 20 hr/week for ≥ 6 months/year. In Maryland the study subjects had to work on study estuaries (Tangier Sound, Smith Island, Cambridge) for ≥ 10 hr/week for ≥ 5 months/year, and in

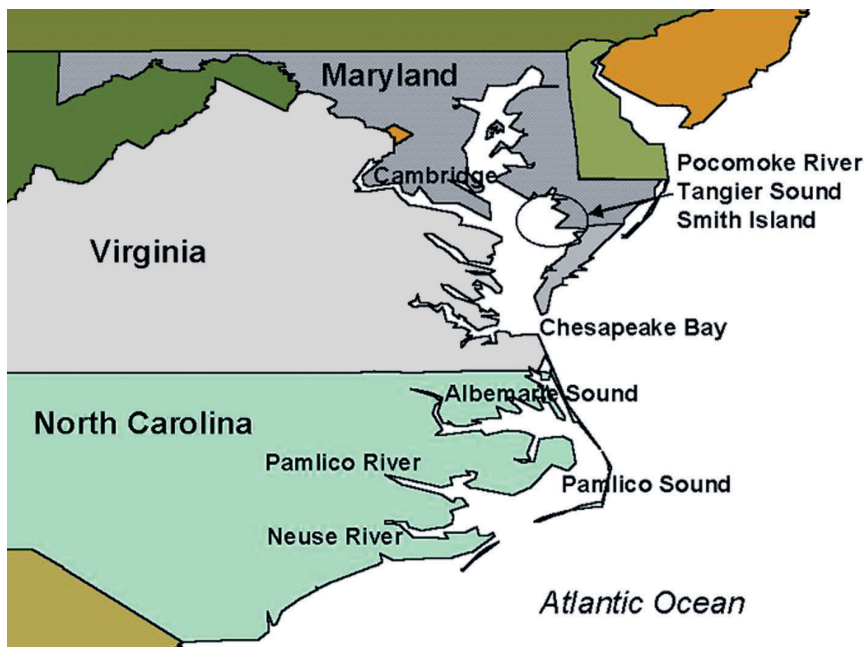


Figure 1. Study areas on the east coast of the United States.

Virginia, study subjects were required to work or engage in recreation on the Chesapeake Bay or its tributaries for >8 hr/week. Individuals with health conditions known to affect neurocognitive function were excluded from the study. Specific medical conditions resulting in exclusion included trauma resulting in loss of consciousness, insulin-requiring diabetes (North Carolina and Maryland only), hospitalization or treatment for drug or alcohol abuse, stroke or transient ischemic attack, brain tumor, seizures, epilepsy, encephalitis, meningitis, Parkinson's disease, Alzheimer's disease, dementia, systemic lupus, Lyme disease, brain surgery, Huntington's disease, multiple sclerosis, narcolepsy, known solvent or pesticide poisoning, reported infection with human immunodeficiency virus (HIV) (North Carolina and Maryland only), head injury, antipsychotic or antimanic medication use, or a diagnosis of psychosis. In addition, individuals who had been placed in mentally handicapped classes in school were excluded from the cohort in Maryland and North Carolina. The North Carolina study also excluded individuals who had participated in previous or other current studies of PEAS.

Baseline and Follow-up Evaluations

At the time of recruitment the study protocol was explained to the study subjects by the project staff and informed consent was obtained prior to the baseline medical and neuropsychological assessment. The medical component includes a full medical history, questions about alcohol and drug use, exposure to solvents and

other hazardous substances, and a physical exam with neurological and dermatological assessments. Serum and urine are collected from each subject and archived for future examination. In Virginia, routine laboratory tests are run as part of each examination.

All three states use a similar panel of neuropsychological tests chosen by a team of neuropsychologists. The neuropsychological evaluation includes a 90-min neurocognitive battery, similar to that used in previous studies of PEAS (11,12), which examines several cognitive domains, including attention and concentration, complex information processing, learning and memory, language, and visual-constructional skills. These tests are conventional, validated measures of neurocognitive performance that have been used in other environmental health studies of neurotoxic effects in adults (14). The neuropsychological measures were selected considering the longitudinal design of the studies. Practice and potential ceiling effects were addressed through the selection of specific measures and use of alternate forms when necessary and will also be controlled for in the analyses through regression modeling. An assessment of personality and mood, demographic questions, and an assessment of confounders are also included in this evaluation. In addition, North Carolina and Virginia administer a computer-based series of tests (neuropsychological evaluation system) that have been used in previous studies of occupational exposures (14).

Vision is evaluated with a series of tests because of evidence in a previous North

Carolina study that suggested that VCS may be adversely affected in estuary fishermen with possible exposure to *Pfiesteria* spp. (13). Subjects in all three states are tested for visual acuity and contrast sensitivity as described previously (13). North Carolina and Virginia also assess color blindness and hue discrimination using standard methods (15,16). Vibrotactile perception threshold is measured in the North Carolina cohort using a portable biothesiometer (R. Fortier Ceramics Registered, Montreal, Canada) because of previous reports that this is a useful technique for detecting peripheral nervous system disease or dysfunction that may result from exposure to neurotoxic substances (17,18).

Routine follow-up evaluations are performed twice a year and are similar to the baseline evaluation. In Virginia all follow-up evaluations are scheduled over two weekends in the fall and two weekends in the spring. In Maryland and North Carolina, follow-up evaluations are done on a rotating basis, depending on the time the subject was recruited into the study.

Study subjects are requested to undergo additional trigger evaluations when certain criteria are met (Table 1). Symptom-based trigger evaluations are performed when a study subject reports new symptoms that are consistent with the PEAS criteria and that cannot be attributed to other causes. Exposure-based trigger evaluations are performed when the study subject was in a zone before, during, or after a fish kill. In Maryland and Virginia the exposure-based trigger evaluations are performed only when the fish kill is believed to be related to *Pfiesteria* spp. In North Carolina, because of uncertainty involved in diagnosing a *Pfiesteria*-related fish kill in a timely fashion, a broad definition of fish kill exposure is used and includes all fish kills reported by the state or self-reported by the study subject. Exposure to fish populations where more than 50% of the fish had sores is also considered a basis for a trigger evaluation in the North Carolina study. The third set of criteria for a trigger evaluation is a combination of symptoms and exposure. In North Carolina and Virginia, if a cluster of three or more subjects reports similar acute symptoms that are compatible with the PEAS criteria (4,5) and include at least one neurocognitive symptom and these individuals have geographically and temporally related exposures, then a trigger evaluation is performed.

Monitoring Health Effects and Exposure

All three states require scheduled monitoring of health effects and environmental exposures in the study subjects. In North Carolina, telephone interviews are conducted every week during the fishing season and every fortnight

Table 1. Criteria for trigger evaluations.

	Maryland	North Carolina	Virginia
Symptom-based evaluations	New report of confusion, Problems with memory or concentration, or new symptoms involving 3 or more systems. Reported as moderate or greater.	New report of confusion, problems with memory or concentration, reported as moderate or severe, duration ≥ 3 consecutive days. Not chronic.	Unexplained symptoms fitting PEAS criteria
Exposure-based evaluations	<i>Pfiesteria</i> -related FK. Subject worked in FK zone 7 days prior or after FK report.	a) State-reported FK. Subject worked in FK zone or adjacent zones 7 days prior to or after FK report. b) Self-reported FK or $\geq 50\%$ fish with sores.	State-reported FK with good DO and high PLO counts
Exposure- + symptom-based evaluations	Additional testing based on trigger criteria or work in confirmed <i>Pfiesteria</i> -related FK area	3 or more subjects report similar acute symptoms (including at least 1 neurocognitive symptom) and have geographically and temporally related exposures.	

Abbreviations: DO, dissolved oxygen; FK, fish kill; PLO, *Pfiesteria*-like organisms.

during the winter. In Virginia, the telephone interviews are conducted every fortnight throughout the year. In Maryland, study subjects complete and mail in study forms on a biweekly basis, with telephone follow-up as needed. Subjects are queried about a variety of symptoms (those included in the PEAS criteria as well as other general health symptoms), how much time (days, hours) they spent on the water, and the type of water-related activities they had engaged in during the past 1–2 weeks. In all three states, study subjects were given maps of the study estuaries that were marked into defined geographic zones with a code assignment. In the written questionnaires or telephone interviews, the subjects report the specific zones where they spent time during the past 1–2 weeks.

Because exposure to substances such as fuel fumes, solvents, and pesticides can cause some of the symptoms included in the PEAS definition, subjects are queried about the type of boat they use and about activities that involve chemical exposure (painting, fiberglass repair, furniture refinishing, etc.) both during the baseline and follow-up evaluations (all three states) and during the telephone interviews (North Carolina and Maryland only).

All three states routinely collect a variety of water quality data on estuaries and rivers in the areas where the cohorts work and recreate. This includes physical measurements, such as salinity and dissolved oxygen; chemical data such as nitrates, chlorophyll a, biochemical oxygen demand; and microbial data on indicators of fecal contamination, such as coliforms and *Escherichia coli*. In addition, all three states attempt to measure the presence and concentration of *Pfiesteria* spp. and *Pfiesteria*-like organisms by light microscopy cell counts and polymerase chain reaction (PCR) (19) and the presence of toxin-producing *Pfiesteria* spp. by fish bioassay (8). Information on general phytoplankton species abundance and distribution is also collected.

Analytical Approach

In each state the data sets with the environmental quality data, the subject exposure information (including location and duration of water-related activities), subject symptom reports, health evaluations, and neuropsychological test performance are merged. Because it is not possible to measure the water quality that each member of the study cohorts is exposed to on a daily basis, exposure to waterborne agents is estimated by matching water quality data from the closest locations and times to those reported by the study subjects.

The general analytical approach for assessing the association between exposure to estuarine water and health effects in the study subjects has been to conduct a series of comparisons of the health outcome measures for groups of subjects with different exposures. Health outcomes have been compared as follows:

- Study subjects exposed to Estuary A versus study subjects exposed to Estuary B.
- Study subjects exposed primarily to estuaries versus those exposed to ocean waters.
- Study subjects exposed to fish kills versus those not exposed to fish kills.
- Study subjects with intense water exposure (based on duration and type of fishing) versus those with little water exposure.
- Study subjects exposed to waters where *Pfiesteria* spp. were recently or currently detected versus those exposed to waters where *Pfiesteria* spp. have never been detected.
- Study subjects exposed to a confirmed *Pfiesteria*-associated fish kill versus those not exposed to a *Pfiesteria*-associated fish kill.

In addition to comparing health outcome measures between exposure groups, changes within specific groups are examined over time, such as health status at the beginning versus the end of the fishing season. The health outcomes compared include general health at medical evaluations, frequency and severity of

reported symptoms, neuropsychological test performance, vision test performance, and other health measures. Education and age are treated as covariates in all analyses. Because it is possible that there may be a differential effect of exposure on participants from different backgrounds, attention is given to clarifying these potential differential effects during the analyses.

Results

Cohort Characteristics

The study cohorts ranged in size from 118 to 238 subjects and are composed of commercial watermen, state workers involved in water sample collection and other coastal activities, researchers engaged in environmental studies, and some community controls (Maryland and Virginia). The cohorts are made up primarily of white males between 30 and 65 years of age. The age distribution of the cohorts is similar in all three states (Table 2). However, the Virginia cohort has a greater proportion of subjects with more than 16 years of education than the other two states because of the inclusion of environmental researchers.

Preliminary Results for the Maryland Study

The cohort study in Maryland identified four exposure groups as follows: Tangier Sound and Smith Island watermen (potential high *Pfiesteria* exposure), Cambridge watermen (predicted low *Pfiesteria* exposure), and community controls (unexposed). The preliminary analyses of data collected in 1998 and 1999 did not indicate significant differences in neurocognitive performance among these four groups or within groups when comparing baseline test performance to end-of-season performance.

Analyses of water samples collected in the study areas indicated that *Pfiesteria* spp. were detected infrequently in the study areas in 1998 and 1999. PCR monitoring for *Pfiesteria* was started in 1999 and has proved to be a sensitive method to detect the organism but does not measure the presence of a toxin. In 1999, 41 of 740 (5%) samples were positive for *Pfiesteria* spp. Most *Pfiesteria* spp. detected were in Fishing Bay and Middle River. Additional *Pfiesteria* detections occurred in the Pocomoke Sound and Manokin River. In 2000 *Pfiesteria* spp. were detected earlier in the summer season and at more locations than in 1999. No *Pfiesteria*-associated fish kills have occurred in the study area since the study began.

Preliminary Results for the North Carolina Study

As of September 2000 the North Carolina study had completed 202 person-years of observation. A total of 238 baseline

Table 2. Demographic characteristics of the three cohorts.

	Maryland (%)	North Carolina (%)	Virginia (%)
Number enrolled	136	238	118
Number withdrawn	14 (10)	15 (6)	12 (10)
Male	134 (99)	207 (87)	97 (82)
Caucasian	136 (100)	235 (99)	108 (92)
Age			
18–29 years	15 (11)	33 (14)	14 (12)
30–41 years	37 (27)	71 (30)	34 (29)
42–53 years	43 (32)	76 (32)	37 (31)
54–70 years	41 (30)	57 (24)	33 (28)
Education			
<12 years	33 (24)	74 (31)	18 (15)
12 years	68 (50)	95 (40)	37 (31)
>12–16 years	19 (14)	48 (20)	28 (25)
>16 years	16 (12)	21 (9)	35 (30)

evaluations, 209 routine follow-up evaluations, 3 symptom-based trigger evaluations, and 117 fish kill trigger evaluations were performed between April 1999 and September 2000. In 1999 there were 7 state-reported fish kills in the study area and 22 fish kills reported by study subjects. Of the 24 fishermen who met the criteria for exposure to a fish kill, 51% completed a fish kill trigger evaluation. In 2000, there were 18 state-reported fish kills in the study area and 62 fish kills reported by study subjects, and 80% of the exposed fishermen completed a fish kill trigger evaluation. None of the state-reported fish kills in 1999 and 2000 were confirmed to be associated with *Pfiesteria* spp. The North Carolina study started 1 year later than the Maryland and Virginia studies, and associations between exposure and health outcomes have not yet been examined.

The North Carolina cohort worked in a wide range of coastal environments in the northern half of the North Carolina coastline. Based on information collected during the weekly telephone interviews from April through September 2000, 37% of the cohort fishing days were in estuaries where *Pfiesteria*-associated fish kills had been reported in previous years, 55% of cohort fishing days were in waters where no *Pfiesteria*-associated fish kills have been reported and *Pfiesteria* exposure may be less likely, and 6% of cohort fishing days were in the open ocean where *Pfiesteria* exposure was unlikely (Table 3). The North Carolina cohort was involved in a variety of fishing activities, including shrimping, clamming, and oystering, but the primary activities were crabbing (52% of fishing days) and fin fishing (25% of fishing days). Fishing activities and other activities reported by the cohort included exposure to chemicals and solvents. Most of the cohort reported cleaning their equipment with degreasers or gasoline (71%), painting or varnishing their

Table 3. Reported fishing locations of North Carolina cohort: April and September 2000.

Geographic area	Characteristics of area	Fishing days (%)
Pamlico and Neuse Rivers	Estuaries with previously reported <i>Pfiesteria</i> activity	37
Albemarle Sound	Estuary with no previously reported <i>Pfiesteria</i> activity. Predicted low risk of <i>Pfiesteria</i> exposure.	14
Atlantic Ocean	Open ocean. Predicted low risk of <i>Pfiesteria</i> exposure.	6
Pamlico Sound	Open water region between barrier islands and mainland. <i>Pfiesteria</i> activity has not been reported, but risk of <i>Pfiesteria</i> exposure has not yet been characterized.	26
Morehead City	Range of coastal environments. Risk of <i>Pfiesteria</i> exposure has not yet been characterized.	15

boats (66%), doing fiberglass repairs (64%), and using insecticides (50%) in the past year. When study subjects were queried about the occupational hazards they were most concerned about, skin cancer and skin problems (such as cuts that did not heal) were the primary health concern. About 75% of study subjects reported that they were somewhat or very concerned about possible health risks associated with *Pfiesteria* spp.

Preliminary Results for the Virginia Study

Between spring 1998 and fall 2000, the Virginia study completed 225 person-years of observation. No subjects with symptoms that met the PEAS surveillance criteria established at the CDC-sponsored workshops were identified in 1998, 1999, or 2000. No significant change in neurocognitive test performance over time was observed for any study subject. In 1998 decreased VCS was observed in the study group exposed to tributaries with constant levels of *Pfiesteria*-like organisms (but not *Pfiesteria* spp.) (20). Preliminary regression analyses indicated that subject age, time spent in waters with *Pfiesteria*-like organisms, and cigarette smoking were significant predictors of decreased VCS. Subsequent Mancova, generalized estimating equations, and regression analyses on the larger data set in 1999 indicated that only age was a significant predictor of decreased VCS ($p < 0.01$). There were five fish kills in 2000, all of which occurred in areas with increased counts of *Pfiesteria*-like organisms in previous years. However, no *Pfiesteria* spp. were associated with these kills, and additional trigger evaluation of study subjects was not done. In addition, there were two algal blooms in the study area, but no *Pfiesteria* spp. were identified in these

blooms. None of the Virginia study subjects has reported any illness that would trigger an additional evaluation.

Discussion

Exposure to *Pfiesteria* spp. has been linked to a variety of clinical symptoms (1,2). Neuropsychological symptoms including new or increased forgetfulness and abnormal scores on some neuropsychological tests have been reported in humans (11,21). Skin lesions or a burning sensation of skin on contact with water have also been reported (11,22). However, to date, the constellation of symptoms potentially associated with exposure to *Pfiesteria* spp. has not been fully defined, and our understanding of the link between human illness and exposure to *Pfiesteria* and related toxic species in estuary waters is extremely limited.

To further elucidate the potential clinical illness associated with exposure to *Pfiesteria* spp. and/or estuarine waters, cohort studies with shared goals and similar, but not identical, methods are being conducted in North Carolina, Virginia, and Maryland. The cohorts are composed primarily of commercial fishermen who have the most exposure, both in terms of duration and direct contact, to estuarine water, and who are most likely to be affected by hazardous substances in the water. The study design permits the examination of multiple health outcomes, and it is possible to control for the effects of individual risk factors such as alcohol consumption or exposure to other hazardous substances. Repeated neurocognitive and medical evaluations and careful monitoring of health symptoms over the 2- to 5-year follow-up period allows the assessment of both severe, acute conditions and more subtle, chronic health effects.

These studies were prompted by concern about human exposure to the dinoflagellate *Pfiesteria* but may be limited in their ability to measure health risks due to *Pfiesteria* spp. and related toxic species for several reasons. First, the presence of toxic *Pfiesteria* in the environment is likely to be a transient event that makes detection and accurate exposure assessment difficult. Second, methods to detect toxin activity are in experimental stages and are not suited for environmental screening. Third, it is currently not possible to directly measure human exposure to the purported *Pfiesteria* toxin via a biomarker. Fourth, despite a combined cohort of more than 400 subjects, these studies may have limited power to assess the large number of potential variables in an analytical model. For some analyses, differences in the study methods between the three studies may restrict our ability to perform a combined analysis with pooled data from all three studies. Finally,

there have been no indications of significant toxic *Pfiesteria* activity in Maryland, Virginia, and North Carolina since these studies began in 1998. Our approach has been to use various surrogate indicators of exposure such as exposure to estuarine water, exposure to waters with *Pfiesteria*-like organisms detected by light microscopy or PCR, or exposure to fish kills. We recognize the serious limitations of our exposure assessment due to temporal and geographic limitations on water quality sampling and analysis and our reliance on self-reported water exposure (duration, nature of exposure, and locations). These studies have also tried to examine self-reported exposures to chemical hazards that may act as confounders.

The preliminary results from all three cohort studies do not indicate detectable adverse health effects in any of the study groups. However, data collection and analyses are ongoing. In the absence of clearly documented *Pfiesteria*-associated fish kills in the study areas, it is possible that none of the study subjects have been exposed to toxic *Pfiesteria* spp. The ongoing investigations may be able to examine the effects of exposure to *Pfiesteria*-associated fish kills in the next study year. To date, the data collected by these studies on three different cohorts of adults with diverse environmental exposures

provide valuable information on the range of neurocognitive performance and medical conditions that is normal for these groups and the degree to which these outcomes change over time. These data will serve as a comprehensive benchmark of neurocognitive performance and health conditions in populations most likely to be exposed to *Pfiesteria* spp. and other hazardous agents in the estuarine environment and against which future groups can be compared in the event of toxic *Pfiesteria* exposure. Finally, there is increasing recognition that there are at least two *Pfiesteria* spp. (23,24) and other *Pfiesteria*-like organisms and that these organisms can be detected in many of the environments under study. Failure to document neurocognitive deterioration in our study subjects may provide reassurance that chronic, low-level exposure to these organisms does not result in detectable health problems. However, further analyses of the final data sets from all three studies are needed to more fully address this environmental health issue.

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Appendix. Acknowledgments

Maryland Study Team

Maryland Department of Health and Mental Hygiene: R. Venezia
Biostatistician: R. Hebel
Neuropsychology: C. Fishburn
Coordinators: J. Young, M. Lawson, P.J. Twilley

North Carolina Study Team

Field team: D. Boyce, V. Dixon, J. Hudson, S. Kuhn, B. Lee, J. Rafson, M. Turman, R. Vickers
Chapel Hill staff: M. Deluca, S. Wilson, C. Mackey, and Battelle Survey Associates
Co-investigators: J-D. Fine, C. Hall, K. Hudnell, D. Loomis, D. Otto, K. Robertson, P. Stewart, M. Swinker
Consultant: J. Rudek

Virginia Study Team

Field staff: D. Belcher, L. Newman
Virginia Department of Health: S. Jenkins, M. Monti, L. Peipins, Eastern Shore Health District and Three Rivers Health District staff
Medical/Neuropsychology staff: J. Taylor, R. Swerdlow, T. Kerkering, S. Lavoie, M. Scott, L. Sharpe, J. Barth
Consultants: K. Hudnell, M. Johnson

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