# Estuary-Associated Syndrome in North Carolina: An Occupational Prevalence Study

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Atlantic coast estuaries recently have experienced fish kills and fish with lesions attributed to *Pfiesteria piscicida* and related dinoflagellates. Human health effects have been reported from laboratory exposure and from a 1997 Maryland fish kill. North Carolina has recorded *Pfiesteria*-related fish kill events over the past decade, but human health effects from environmental exposure have not been systematically investigated or documented here. At the request of the state health agency, comprehensive examinations were conducted in a cross-sectional prevalence study of watermen working where *Pfiesteria* exposure may occur: waters where diseased or stressed fish were reported from June to September 1997, and where *Pfiesteria* had been identified in the past. Controls worked on unaffected waterways. The study was conducted 3 months after the last documented *Pfiesteria*-related fish kill. The goal was to document any persistent health effects from recent or remote contact with fish kills, fish with lesions, or affected waterways, using the 1997 U.S. Centers for Disease Control and Prevention case description for estuary-associated syndrome (EAS). Examinations included comprehensive medical, occupational, and environmental history, general medical, dermatologic, and neurologic examinations, vision testing, and neuropsychologic evaluations. Seventeen of 22 watermen working in affected waters and 11 of 21 in unaffected waters reported exposure to a fish kill or to fish with lesions. We found no pattern of abnormalities on medical, neurologic, neuropsychologic, or NES-2 evaluation. By history, one subject in each group met the EAS criteria, neither of whom had significant neuropsychological impairment when examined. Watermen from affected waterways had a significant reduction in visual contrast sensitivity (VCS) at the midspatial frequencies, but we did not identify a specific factor or exposure associated with this reduction. The cohorts did not differ in reported occupational exposure to solvents (qualitative) or to other neurotoxicants; however, exposure history was not sufficiently detailed to measure or control for solvent exposure. This small prevalence study in watermen, conducted 3 months after the last documented fish kill related to *Pfiesteria*, did not identify an increased risk of estuary-associated syndrome in those working on affected waterways. A significant difference between the estuary and ocean watermen was found on VCS, which could not be attributed to any specific factor or exposure. VCS may be affected by chemicals, drugs, alcohol, and several developmental and degenerative conditions; it has not been validated as being affected by known exposure to dinoflagellate secretions. VCS should be considered for inclusion in further studies, together with documentation or quantification of its potential confounders, to assess whether it has utility in relationship to dinoflagellate exposure. *Key words*: dinoflagellate, marine toxin, memory disorders, occupational disease, *Pfiesteria*, visual contrast sensitivity (VCS). *Environ Health Perspect* 109:21–26 (2001). [Online 30 November 2000]
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In the decade since its identification, the toxic dinoflagellate *Pfiesteria piscicida* has been implicated in fish kills and fish ulcers in North Carolina, affecting primarily the estuaries of the Neuse and Pamlico Rivers (*1,2*). This organism and related dinoflagellate species have been implicated in fish kills/disease from the mid-Atlantic to the Gulf coast. *Pfiesteria* has a complex life cycle, including over 20 flagellated, amoeboid, and encysted forms (*3,4*). The presence of fish excreta/secretions in water triggers encysted cells to emerge in zoospore form, capable of excreting a bioactive substance that causes lethargy and skin injury in fish. The organism can then feed on the affected

fish; other forms feed on algae and do not elaborate toxic substances. Fish ulcerations may be caused by multiple factors, so the presence of fish lesions alone is not a reliable indication of toxic *Pfiesteria* (*5*). A significant fish kill and errant fish behavior, in the presence of the dinoflagellate, are somewhat more specific. Current techniques can document the presence of dinoflagellates and their potential to be induced to kill fish under laboratory conditions (*6*).

Prolonged, repeated human exposure to fish cultures in a laboratory setting has been reported to cause adverse effects (*7*). Three researchers working with toxic cultures of *Pfiesteria* for weeks to months reported

symptoms including mucous membrane and skin irritation, headache, cognitive problems, fatigue, paresthesias, and gastrointestinal complaints. Skin and/or aerosol contact was postulated as the exposure route. Most symptoms were transient but some persisted. Neuropsychologic testing in one case was consistent with an organic deficit with an amnestic syndrome, which normalized after 2 months.

Following reports of symptoms in persons exposed to fish kills, epidemiologic investigations were conducted in North Carolina. A population survey did not identify a pattern of chronic health problems or neurologic symptoms reported by crabbers on affected waters, compared to crabbers elsewhere, but did document some of their work practices (*8*). Due to methodologic limitations, an investigation of persons exposed to fish kills could not clearly link reported health effects with this contact (*9*). The investigator reported that at one fish kill site, symptom onset was associated with a hydrogen sulfide-like smell, as might be released from the fish carcasses.

In August 1997 a large fish kill occurred on the Pocomoke River in Maryland which was eventually attributed to *Pfiesteria* and at least one other dinoflagellate. Twenty-four

people who had direct contact with the affected waters during a fish kill or with fish that had lesions were assessed acutely (*10*). Exposed subjects reported symptoms of confusion/forgetfulness, headache, skin lesions, and skin burning. When 19 subjects were tested soon after exposure, unexplained and significantly decreased performance resulted on three neuropsychologic tests that measured learning: the Rey Auditory Verbal Learning Test (RAVLT), response inhibition, and fine motor coordination and dexterity. When additional subjects were recruited later via hotline, a significantly elevated odds ratio for abnormalities on the RAVLT was observed in those who had fished or had directly handled fish with lesions; no such association was found in subjects who had been swimming or boating (*11*). Dermatologic examination revealed many skin lesions in the index cases, but most were due to common dermatoses or unrelated conditions. Five individuals had unexplained skin lesions biopsied, which showed "variable patterns of inflammation suggesting reactive erythema, allergic, toxic, or eczematous reactions" (*12*). Acute skin burning which began immediately on contact with water was reported to resolve within 12 hr (*10*); most other acute complaints subsided over a week. Cognitive problems were more persistent; confusion occurring within 12–36 hr of exposure improved gradually over 10–12 weeks. By 3–6 months after exposure, subjects had returned to their presumed baselines on neuropsychologic testing (*13*).

Neuropsychologic assessment and computer-based testing can provide objective evidence of subclinical behavioral or neurologic changes not detectable on physical examination, before development of overt peripheral neuropathy or encephalopathy (*14*). Results are typically compared to established norms, if the study population is closely comparable to the normative population, or to control groups. Neurotoxin exposure can affect attention, executive function, short-term memory, and visual-spatial ability (*15*). Specific tests such as the RAVLT are sensitive to neurotoxin-related deficits (*16*). The Neurobehavioral Evaluation System (NES-2), a computerized testing system, is an accepted standard for occupational neurobehavioral testing (*17*). Subclinical alterations in ocular function are common with chemical exposures (*18*) and may manifest as changes in central vision, color vision, contrast sensitivity, or visual evoked potentials. Visual contrast sensitivity (VCS), a measure of the ability to detect visual patterns, can be a sensitive indicator of subclinical neurotoxicity from a variety of agents (*19*). Deficits in VCS have been observed with occupational exposure to neurotoxic agents such as solvents and heavy metals as well as with some congenital and degenerative neurologic conditions (*15,18,19*). Sensitive but nonspecific, VCS may have utility as a screening test and was included with the NES-2 as an accepted core test for environmental health field studies (*16*). While VCS abnormality is not generally diagnostic of any particular disease state or exposure, some neurotoxicants affect VCS in a characteristic frequency pattern (*18,19*).

In October 1997, the Centers for Disease Control and Prevention (CDC), collaborating with affected states and other federal agencies including the U.S. Environmental Protection Agency (U.S. EPA) and the National Institute for Occupational Health and Safety (NIOSH), formulated a case description for estuary-associated syndrome (EAS) (*20*). It included exposure criteria—exposure to estuarine water characterized by a fish kill and/or fish with lesions (involving 20% of a sample of at least 50 fish of one species) or to affected fish without lesions if *Pfiesteria* or morphologically related organisms (MRO) is suspected; and clinical symptoms—memory loss or confusion, or acute skin burning on water contact, or three or more other symptoms as listed in Table 1. The Maryland group retrospectively noted that these criteria have a negative predictive value of 93% but a positive predictive value of 44% for neuropsychologic deficits on the RAVLT (*13*).

## Methods

The North Carolina state health agency in 1997 approached the U.S. EPA and the Schools of Medicine at Duke University, East Carolina University, and the University of North Carolina at Chapel Hill to perform evaluations for persistent health effects in a group of watermen with full-time occupational exposure to affected waterways (*21*).

Examinations were conducted in November 1997, 3–4 months after the last recognized fish kill involving *Pfiesteria*, a late July event moderate in size (*22*). At that time, long-term follow-up of Maryland subjects had not been completed; the possibility of long-lasting effects was still in question. Questions about chronic exposures or cumulative effects had been raised based on the prior reports (*7,10*). The timing of the evaluations meant that examinations would detect chronic or persistent health effects only; a past history of symptoms could be recorded but not objectively verified. Subjects were identified and recruited by the state health agency in conjunction with other state agencies.

The occupationally exposed cohort was recruited from a roster of licensed commercial fishermen acquired from the North Carolina Division of Marine Fisheries. Potentially exposed subjects were recruited from among commercial gill net and crab pot fishermen working the Neuse and Pamlico Rivers and tributaries, and from among state workers with similar exposure, based on a mapping of locations where diseased or stressed fish and/or validated fish kills had been reported from June to September 1997 (*2,21*). This area roughly corresponded to confirmed toxic *Pfiesteria* sites in prior years (*21*), and hereafter is referred to as "risky waters." Study subjects were considered at risk due to suspicion of past contact or ongoing exposure to risky waters. In the absence of a reliable environmental marker for exposure to dinoflagellate toxin, time spent on risky waters was used as a surrogate exposure index for potential *Pfiesteria* or exposure to MRO.

Twenty-three individuals who worked on the Pamlico and Neuse waterways were recruited, representing a convenience sample of the first 20 persons who could be contacted by telephone and who completed an

**Table 1.** CDC consensus criteria for possible *Pfiesteria* or PLO-related illness or EAS.

| A. Exposure criteria | Exposure to estuarine water characterized by one of the following: |
|---|---|
| | 1. Fish with lesions consistent with *Pfiesteria piscicida* or MRO toxicity with 20% of at least 50 fish of one species having lesions.[a] |
| | 2. A fish kill with lesions consistent with *Pfiesteria* or MRO toxicity. |
| | 3. A fish kill involving fish without lesions, if *Pfiesteria* or MROs are present and there is no alternative reason for a fish kill. |
| B. Clinical symptoms | Reporting of one or more of the following signs or symptoms: |
| | 1. Memory loss |
| | 2. Confusion |
| | 3. Acute skin burning (upon direct contact with water) |
| | 4. Or three or more of the following: |
| | Headaches |
| | Skin rash |
| | Eye irritation |
| | Upper-respiratory irritation |
| | Muscle cramps |
| | Gastrointestinal complaints (including nausea, vomiting, diarrhea, and/or abdominal cramps) |

Adapted from Results of the Public Health response to *Pfiesteria* workshop B (*20*).
[a]For this study, identification of species and/or percentages was not required.

occupational practices survey to verify eligibility; subjects who agreed to participate were invited to do so during that call. For study purposes, fishermen in 18–38 ft (midsize) vessels working full-time on these waters during the warm weather months were considered eligible. In 1997, there were 822 active commercial licenses for the Neuse and Pamlico areas for all vessel sizes (*23*). Some individuals held more than one license, one for fin fishing and one for crabbing.

Based on the 1997 distribution of vessel sizes on the Neuse and Pamlico, approximately 300–350 individuals held licenses for midsize vessels. It is not known how many of these met the occupational activity criteria to be considered a potential study subject, i.e., how many were actively fishing full-time there during the summer months. In addition to the full-time gill net or crab pot fishermen, four were state employees with duties working on these waterways (Table 2). Matches for unexposed male fishermen were selected from the licensing data base for watermen of the Outer Banks, coastal islands distant from the affected estuaries and tidal rivers. The controls used vessels comparable in size to those of the exposed group. Matches for the four exposed female fishermen could not be located; three women with past commercial fishing experience and one with exposure from seafood handling were recruited. Approximately 150 phone calls were made to recruit the 45 subjects.

Matches for the exposed state employees were county and state employees of various occupations. The nonexposed cohort successfully matched the exposed in age (± 3 years), sex, and education (± 2 years), with three exceptions for age or education and the occupational compromises noted. Air transportation was provided for offshore subjects because of lengthy travel times from the Outer Banks. All subjects received a monetary stipend for participating.

The comprehensive multidisciplinary evaluations included standardized medical, occupational/environmental, neurobehavioral, and neurologic histories. General medical, dermatologic, and neurologic examinations and vision screening were conducted and recorded in a standardized form. Selected elements of the NES-2 computer-based testing system were administered. Visual contrast sensitivity (VCS) was tested using the Functional Acuity Contrast Test (FACT) card (*19*). The neuropsychologic test battery was comprised of standardized instruments assessing performance across neurocognitive domains: learning and memory, complex information processing, language, visual-constructional abilities, and fine motor skills. The evaluation procedure lasted 6 hr for each subject.

The neuropsychologic tests were chosen after discussion among neuropsychologists from Maryland, Virginia, and North Carolina, but the inclusion of NES-2 and vision testing was unique to this study. Control subjects were included from the outset to aid in interpretation of the neuropsychologic and vision testing. All examiners were blinded to the status of the subjects to minimize the potential for bias. Informed consent was obtained from the subjects as part of the Institutional Review Board protocol. Blood and urine specimens were collected, and a standard multiphasic biochemical/hematologic panel was performed. Aliquots of urine and serum were frozen and sent to CDC for storage pending development of biomarkers. When a subject was found to have a significant and previously unrecognized condition, a letter was sent to his or her personal physician if prior consent had been given.

A standardized neurologic examination format was adapted from a widely used clinical classification system (*24*), incorporating history and examination data into scores for such individual parameters as mental status, cranial nerve, sensory, and motor or cognitive function, and into an overall summary rating for "neurotoxic complex staging" (Appendix). Neuropsychologic test results were converted to standard scores and percentiles using published normative data. Two neuropsychologists, blind to exposure and confounder status, reviewed the data and assigned clinical ratings by consensus: "0" indicated within normal limits; "1" borderline to mild neuropsychologic weakness; "2" significant neuropsychologic impairment. Both the clinical ratings and mean test scores were used in comparisions between groups. NES-2 elements included digit span, finger tapping, simple reaction time, switching attention, and symbol-digit substitution tests. Medical examination data were coded by a physician; the NES-2 and vision data were analyzed by experts from the EPA. For VCS, data from an eye were excluded if corrected visual acuity was less than 20:70, to avoid confounding by excessive refractive error. Processed data were submitted to the University of North Carolina School of Public Health for compilation into a preliminary report to the Department of Health and Human Services.

The exposed and unexposed cohorts were examined to verify the appropriateness of their exposure classification. A potential "exposure" activity was defined as activity on the water occurring within 5 miles of a validated fish kill or distressed/diseased fish sighting. A "nonexposure" activity occurred farther than 5 miles from such an occurrence. The choice of 5 miles to define exposure is somewhat arbitrary; dinoflagellate toxin has not been characterized completely and cannot be measured in the environment. There is no capability to detect or track its persistence or spread in the environment. It is known that fish kills can "extend for miles and last for days" (*25*). Watermen with activity conducted over 5 miles from a dinoflagellate event were assumed to be unexposed, but it is less clear that all those working within 5 miles of an event would have any actual exposure. In the absence of data, this approach was taken to maximize sensitivity and detect as many potentially affected persons as possible. An exposure parameter was developed by multiplying, for each job, the number of months worked during the 6 warm-weather months of potential fish kills multiplied by the days per month worked and the number of years on the job. A maximum of 30 days each month could be assigned to such activities. A cumulative statistic was determined for the prior 2 years. For recreational activities a similar approach was used; when the number of days assigned to commercial and recreational exposures exceeded the number of days in a month, the commercial activity was given precedence.

## Results

Of 45 subjects examined, two were dropped from analysis due to lack of an appropriate age-matched control or poor effort on testing. This left 22 individuals exposed to risky waters; 17 were fishermen. They were matched with 21 unexposed controls, offshore watermen with a smaller proportion of active fishermen (Table 2). The exposed individuals worked primarily in areas where fish kills due to dinoflagellates had been reported during both of the prior 2 years. It was not possible to verify that each exposed subject was present at a specific site on the exact date when an incident occurred, but of the 22 exposed watermen, 16 reported exposure to a fish kill and 14 to fish with sores

**Table 2.** Exposure of watermen.

| Current occupation | Exposed (*n* = 22) | Percent | Unexposed (*n* = 21) | Percent |
|---|---|---|---|---|
| Commercial fisherman, crabber | 17 | 77.7 | 11 | 52.4 |
| Marine biologist/environmental specialist | 4 | 18.2 | 4 | 19.2 |
| Boat charter | 0 | 0 | 2 | 9.2 |
| Other water-related occupation | 1 | 4.5 | 1 | 4.5 |
| Recreational exposure, not occupational | 0 | 0 | 3 | 13.7 |

(Table 3). Four of 21 unexposed watermen reported exposure to a fish kill; 3 of these did not involve estuaries or their tributaries. Eight reported exposure to fish with sores.

The exposed and unexposed subjects were similar in age (mean = 41.7, SD = 9.1; and 41.2, SD = 9 .2, respectively) and education (mean = 13.4, SD =2.46; and 14.0, SD = 2.19). A greater proportion of the unexposed were currently unmarried (32% vs. 17%). Nearly all persons in both groups reported engaging in recreational water activities (90%); most (73% and 78%) resided within 1,000 ft of the water. Review of the cumulative exposure statistics suggests that the process of exposure status classification was generally appropriate (Table 4) with minimal "crossover." Significantly, the exposed watermen worked nearly 50% more days on the water than the unexposed.

Qualitative solvent or fume exposure (at least once a week) was reported by 50% of the exposed and 52% of the unexposed; exposure to pesticides, metals, or metallic compounds including lead or mercury was also similar (Table 3). Questions on solvent use did not detail specific work practices or attempt to quantify the intensity, frequency, or route of exposure; e.g., history was not sufficiently detailed to differentiate between gasoline used for skin cleaning versus fuel poured into a gas tank.

Of the 22 exposed watermen, 17 reported exposure to fish kill or fish with lesions. Four had been exposed to the confirmed 1997 *Pfiesteria* bloom and 2 others were possibly exposed to it. Three of the estuary watermen noted at least one acute symptom listed in the EAS complex; none of the three had contact with the July 1997 event. A 38-year-old fisherman reported multisystem symptoms that met the EAS case definition: nasal and eye irritation, gastrointestinal upset, headache, dizziness, confusion, and memory and concentration problems, beginning within a day of exposure and resolving after a week. This subject's neurologic exam was within normal limits; his neuropsychologic performance was mildly below expectation. History was positive for medication use (amitriptyline, sumatriptan), long-standing cervical compression neuropathy, and past head injury. He reported sporadic exposure to dead or lesioned fish every summer for 4–5 years as well as during the current year. The other two subjects reported skin problems only, resolved by the time of examination in one and persistent as eczema since 1995 in the other.

Eleven of the unexposed watermen gave a history of exposure to fish kills/sores, usually as a sporadic occurrence. Of the four who reported exposure to fish kills, two were not in an estuary, and one gave no information on location. The fourth was present at the 1997 fish kill site but stood 1,000 ft away from the shore and had no water contact or symptoms. Eight of the unexposed watermen (38%) had encountered lesioned fish as a sporadic event, not part of the recognized outbreak. One 37-year-old ocean fisherman reported acute symptoms related to handling lesioned sea trout during several weeks in summer 1994, unassociated with a recognized dinoflagellate event. He had no exposure to a fish kill. He reported feeling slightly dizzy or unsteady acutely, without any irritative or other acute symptoms, followed by subsequent problems with memory and forgetfulness. This subject could not recall when these symptoms began in relationship to the exposure, but had normal performance on neuropsychologic testing despite multiple potential confounders including current heavy alcohol use (five or more drinks per day).

Peripheral nerve conditions were not uncommon in the medical histories of the watermen. The exposed watermen included seven (32%) with a long-standing history of neuropathy due to a nerve root or peripheral nerve compression. Among the ocean watermen, four (19%) gave such a history. There were no significant differences between the groups on general medical examination or blood tests, although more exposed watermen were recommended for follow-up for various unrelated conditions (Table 5). Dermatologic examinations did not reveal any pattern of unique or unusual skin lesions, but sun-related skin damage was a frequent finding.

The groups were similar in the frequency of confounding influences on neuropsychologic test performance, such as difficulty in school, serious past or current psychiatric symptoms, daily marijuana use, past or current other drug abuse, or medical conditions potentially affecting cognition. A greater proportion of the exposed indicated a history of neurologic disease (2 vs. 0) and current use of medications with potential cognitive effects (3 vs. 1). Unexposed persons were more likely to report heavy past or current alcohol use (7 vs. 3), and a history of head injury (3 vs. 1).

Signs of peripheral neuropathy (Table 6), based on changes in motor or sensory function or reflexes, were found in eight exposed (36.5%) and four unexposed (19%) individuals. In most cases these were related to a previously recognized medical condition and affected sensory function primarily. Two individuals were rated abnormal on the overall neurotoxic complex staging, one exposed and one unexposed; neither had noted EAS symptoms.

The neuropsychologic test battery generated 28 individual test scores and an overall clinical rating (Table 7), which were compared across groups. Four comparisons measuring simple and complex attention and memory were associated with $p$-values of ≤ 0.10, with the unexposed group performing better than the exposed group. Other tests measuring similar abilities did not show such a pattern. The RAVLT indices typically most sensitive to neurotoxin exposure did not show a significant difference between the groups. The watermen were assigned overall clinical ratings by two neuropsychologists blind to exposure status and confounder history. The pattern of clinical ratings was similar in both groups, and most subjects who received borderline ratings had explanatory factors. Two subjects were free of confounding factors and rated "borderline"; one was exposed and the other was unexposed. Neither had histories consistent with EAS.

If the sample is restricted to subjects without potential confounders for neuropsychologic deficits, four comparisons—measuring simple attention, memory, and learning—were associated with $p$-values ≤

**Table 3.** Exposure of watermen.

| Exposure type | Exposed ($n$ = 22) | Unexposed ($n$ = 21) |
|---|---|---|
| Exposed to solvents/fumes | 11 (50%) | 11 (52.4%) |
| Exposed to metals or pesticides | 5 (27%) | 7 (33.3%) |
| Exposed to fish kill/suspicious for *Pfiesteria* | 16 (72.7%) | 1 (4.8%) |
| Exposed to fish with lesions[a] | 14 (63.6%) | 8 (38.1%) |
| Symptoms consistent with EAS | 1 (4.5%) | 1 (4.8%) |

Odds ratio for EAS symptoms = 0.95 (95% CI = −16.11 to 17.8).
[a]Not necessarily a documented dinoflagellate event.

**Table 4.** Watermen mean cumulative occupational exposure[a] to risky[b] and nonrisky[c] waters.

| Waters | Cohort | |
| | Exposed | Unexposed |
|---|---|---|
| Risky waters mean total exposure days | 281 | 8 |
| Nonrisky waters mean total exposure days | 22 | 172 |

[a]Combined cumulative occupational and recreational exposure days in warm weather months in the past 2 years; maximum = 360 days. [b]Working within 5 miles of a documented *Pfiesteria*-related fish kill or distressed/diseased fish. [c]Working > 5 miles from a documented *Pfiesteria*-related fish kill or distressed/diseased fish.

0.10. The 12 unexposed subjects performed better on the two measures of simple attention, while the 14 exposed subjects performed better on learning and memory. Again, other tests that measure similar abilities did not show the same patterns.

The computerized NES-2 testing battery yielded 13 scales, which were compared between the exposed and unexposed. None of the comparisons produced a $p$-value < 0.22, with exposed subjects showing a small deficit on five scales and a small excess on eight scales. Analysis of the nonconfounded subjects yielded a very similar pattern, with no notable differences on individual tests or overall results.

The estuary and offshore participants were not significantly different in visual acuity or color vision. Mean VCS was lower in the estuary (exposed) than the offshore (unexposed) subjects at all five spatial frequencies, with significant VCS reductions at the midspatial frequencies (6 and 12 cycle/degree). When analysis was repeated on the nonconfounded subjects, group differences in mean VCS were greater at each spatial frequency (19).

## Discussion

Most of the exposed watermen had contact with diseased fish or fish kills and have worked in an area where such occurrences have been attributed to dinoflagellates. No unexposed watermen had direct water contact with a fish kill related to a recognized dinoflagellate event, but sporadic encounters with lesioned fish were reported by about one-third.

In this cross-sectional study, the exposed and unexposed cohorts were similar on general medical examination and on neuropsychologic testing. Peripheral neuropathy was found more often in the exposed cohort but was usually due to a preexisting or recognized condition. One exposed and one unexposed individual had a history of symptoms consistent with EAS. Neither had current abnormalities on neuropsychologic testing. In this very small study, there was no greater risk of EAS by history and no more persistent sequelae on examination in exposed watermen than in unexposed. Given the timing of the evaluations and the small sample size, these results do not rule out the occurrence of previous transient or unrecognized neuropsychologic impairment or the sporadic occurrence of problems with a low attack rate.

Watermen on risky waters spent about 50% more time on the water than did the others, increasing their exposure to any hazard in their working environment. Crabbers comprised the majority of exposed fishermen, so we reviewed their histories separately. Crabbers were more likely to report

solvent exposure (OR = 3.5; 95% CI, 1.1–13.3) and to work in the estuaries (OR = 7.9; 95% CI, 2.23–28.2). Of 26 crabbers, 16 were part of the exposed cohort. Except for one, the exposed crabbers worked full-time on the risky estuary waters. The 9 crabbers in the unexposed cohort crabbed only part of the year and fished primarily in nonrisky waters for the rest of the year. It has been suggested that the work practices of crabbers may involve regular use of petroleum products to "tar" their traps and gasoline for hand cleaning, but this level of specificity was not obtained in history. Hydrogen sulfide has been recognized as a potential for exposure in large-scale fishing and fish-processing vessels (25,26). In the United States a permissible exposure level of 20 ppm averaged over 8 hr applies in industrial settings (26). Limited case reports suggest that exposures below that level can be associated with fatigue, poor memory, dizziness, and irritability, and that community exposures near landfills can lead to headache, eye irritation, and respiratory problems (27). The hydrogen sulfide smell noted at one fish kill site (9) and the work practices described above raise the

possibility that *Pfiesteria* or MRO may not be the only exposure difference between the ocean and estuary fisherman, but these confounding factors require exploration in more depth.

There were significant differences between the groups on VCS. It is not possible to link these findings to a specific exposure. Potential confounders include exposure to other chemicals, to chronic sunlight without UV eye protection, or to unknown factors related to estuaries or marine toxin(s). VCS is a relatively sensitive indicator of subclinical effects deriving from a variety of neurotoxicants and clinical conditions, reflecting dysfunction anywhere in the course of the visual pathway from retina to occipital cortex or, to a lesser extent, in the lens or cornea (28). It can be affected by common exposures such as anticonvulsant medication use or alcohol ingestion to near-intoxication (29). Because norms have not been established to allow its use as a diagnostic test in the individual, VCS has greatest utility in population studies. Its role as a marker for exposure or effect related to dinoflagellate toxin remains to be determined and validated. Because no currently symptomatic cases of EAS were found in this

**Table 5.** Watermen with unrecognized findings significant enough to recommend follow-up.

| Conditions | Exposed ($n$ = 22) | Unexposed ($n$ = 21) |
|---|---|---|
| Preneoplastic skin changes | 3 | 1 |
| Other recognized dermatologic problem | 1 | 0 |
| Psychological distress | 2 | 0 |
| Hyperglycemia | 1 | 0 |
| Neurologic impairment[a] | 1 | 1 |
| Neuropsychologic test results | 1 | 0 |
| Atherosclerotic coronary vascular disease | 0 | 1 |
| Total subjects recommended for follow-up | 8 | 3 |

[a]Lumbar nerve root compression

**Table 6.** Watermen: summary of neurologic examinations.

| Stages | Exposed ($n$ = 22) | Percent | Unexposed ($n$ = 21) | Percent |
|---|---|---|---|---|
| Neurotoxic complex[a] staging | | | | |
| (0) normal | 21 | 95.5 | 20 | 95.2 |
| (0.5) subclinical | 1 | 4.5 | 1 | 4.8 |
| (1) mild | 0 | 0 | 0 | 0 |
| (2) moderate | 0 | 0 | 0 | 0 |
| (3) severe | 0 | 0 | 0 | 0 |
| (4) end stage | 0 | 0 | 0 | 0 |
| Overall peripheral neuropathy | | | | |
| (0) none | 14 | 63.6 | 17 | 81 |
| (1) mild | 8 | 36.5 | 4 | 19 |
| (2) moderate | 0 | 0 | 0 | 0 |
| (3) severe | 0 | 0 | 0 | 0 |

[a]Combines mental status and motor function.

**Table 7.** Watermen: clinical summary ratings of neuropsychologic testing.

| Rating | Exposed ($n$ = 22) | | Unexposed ($n$ = 21) | |
|---|---|---|---|---|
| | Frequency | Percent | Frequency | Percent |
| (0) normal | 17 | 77.3 | 17 | 81 |
| (1) borderline/mild | 5[a] | 22.7 | 4[a] | 19 |
| (2) impaired | 0 | 0 | 0 | 0 |

[a]All but one gave a medical history that could potentially explain their test performance.

study, no relationship between VCS and EAS can be postulated. In the future, should it be demonstrated that VCS is sensitive to dinoflagellate-induced neuropsychologic impairment, this test could have utility as a screening test; however, much additional work remains to be done to assess its value.

In this retrospective evaluation, few watermen reported symptoms that might suggest past history of EAS. One exposed waterman and one unexposed waterman reported symptoms consistent with EAS by history, but neither had significant objective abnormalities on examination. It is not possible to verify a case of EAS in these subjects or to infer an association of symptoms with a particular environmental exposure, acute or chronic. In Maryland acute symptoms occurred and resolved quickly; e.g., skin burning resolved within 12 hr after water contact, and respiratory irritation and headache resolved within 3 days to 1 week (*10*). The neuropsychologic abnormalities began within 1–2 days and resolved over weeks to months (*13*). Because the last recognized *Pfiesteria*-associated fish kill in North Carolina occurred 3 months before the examinations, if North Carolina subjects had been affected, it is expected that all medical effects and most neuropsychologic effects would have resolved, assuming a clinical course similar to that observed in Maryland.

Since 1997 researchers have focused intently on identifying the toxins related to marine exposure and the organisms elaborating them. Characterization of the nature, distribution, and half-life of these bioactive substances in the natural environment and development of methods for their detection in biological or environmental specimens would greatly advance efforts to assess potential human health effects. In the absence of reliable biomarkers of exposure or effect, time and place were used as surrogate exposure indices. Until such markers are available, it will be difficult to assess exposure without relying on such imprecise and nonspecific surrogate measures, which increase the level of uncertainty.

In 1999, the CDC revised the case description of EAS, renaming it possible estuary-associated syndrome (PEAS). The exposure criteria were broadened to include any exposure to estuarine water (*30*). The same spectrum of symptoms is retained with onset expected within 2 weeks of exposure. Contact with dead or lesioned fish is no longer required for PEAS, reflecting uncertainty about such contact's role as an indicator for the presence of any putative toxin. Using this current case description, all the estuary fishermen would meet the exposure criteria for PEAS, and one would meet the criteria for past PEAS by history.

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**Appendix.** Neurotoxic complex staging.

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|---|---|
| Stage 0 (normal) | Normal mental and motor function. |
| Stage 0.5 | Absent, minimal, or equivocal symptoms without impairment (equivocal/subclinical) of work or capacity to perform activities of daily living (ADL). Mild signs (reflex changes, slowed ocular or extremity movements) may be present. Gait and strength normal. |
| Stage 1 (mild) | Able to perform all but the more demanding aspects of work or ADL, but with unequivocal evidence (symptoms or signs, including performance on neuropsychologic testing) of intellectual or motor impairment. Can walk without assistance. |
| Stage 2 (moderate) | Able to perform basic activities of self-care but cannot work or maintain more demanding aspects of daily life. Ambulatory, but may require single prop. |
| Stage 3 (severe) | Major intellectual incapacity (cannot follow news or personal events, cannot sustain complex conversation, considerable slowing of all output) or motor disability (cannot walk unassisted, requiring walker or personal support, usually with slowing and clumsiness of arms as well). |
| Stage 4 (end stage) | Nearly vegetative. Intellectual and social comprehension and output are at rudimentary level. Nearly or absolutely mute. Paraparetic or paraplegic with double incontinence. |

Adapted from Tross et al. (*24*).