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Early report

Learning and memory difficulties after environmental exposure to waterways containing toxin-producing *Pfiesteria* or *Pfiesteria*-like dinoflagellates

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Summary

Background At the beginning of autumn, 1996, fish with "punched-out" skin lesions and erratic behaviour associated with exposure to toxins produced by *Pfiesteria piscicida* or *Pfiesteria*-like dinoflagellate species were seen in the Pocomoke River and adjacent waterways on the eastern shore of the Chesapeake Bay in Maryland, USA. In August, 1997, fish kills associated with *Pfiesteria* occurred in these same areas. People who had had contact with affected waterways reported symptoms, including memory difficulties, which raises questions about the human-health impact of environmental exposure to *Pfiesteria* toxins.

Methods We assessed 24 people who had been exposed. We collected data on exposure history and symptoms, did a complete medical and laboratory assessment (13 people), and carried out a neuropsychological screening battery. Performance on neuropsychological measures was compared with a matched control group.

Results People with high exposure were significantly more likely than occupationally matched controls to complain of neuropsychological symptoms (including new or increased forgetfulness); headache; and skin lesions or a burning sensation of skin on contact with water. No consistent physical or laboratory abnormalities were found. However, exposed people had significantly reduced scores on the Rey Auditory Verbal Learning and Stroop Color-Word tests (indicative of difficulties with learning and higher cognitive function), and the Grooved Pegboard task. There was a dose-response effect with the lowest scores among people with the highest exposure. By 3–6 months after cessation of exposure, all those assessed had test scores that had returned to within normal ranges.

Interpretation People with environmental exposure to waterways in which *Pfiesteria* toxins are present are at risk of developing a reversible clinical syndrome characterised by difficulties with learning and higher cognitive functions. Risk of illness is directly related to degree of exposure, with the most prominent symptoms and signs occurring among people with chronic daily exposure to affected waterways.

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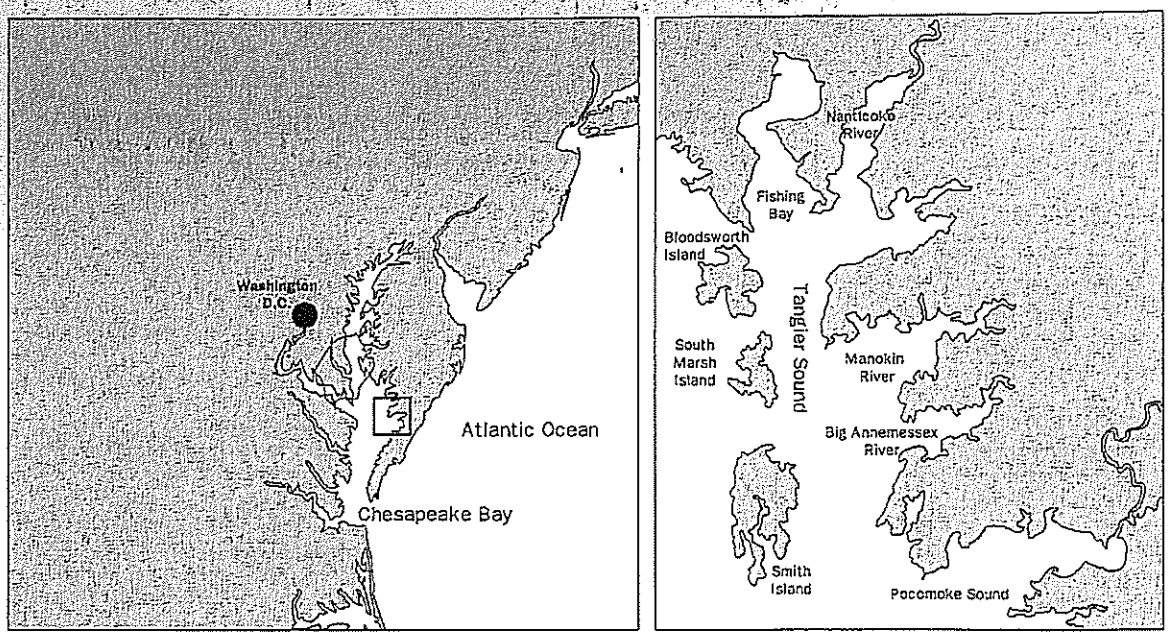
Introduction

In autumn, 1996, commercial fishermen (watermen) began noticing unusual "punched-out" necrotic ulcers and erratic swimming behaviour in fish in the Pocomoke River and nearby estuaries on the eastern shore of the Chesapeake Bay, Maryland, USA (figure). Fish with these lesions were sporadically observed throughout the winter, and were seen in increasing numbers throughout the spring and summer of 1997. In studies by the Maryland Department of Natural Resources, almost all Atlantic menhaden collected in surveys on the Pocomoke River between June 17 and Aug 27, 1997, had these characteristic skin lesions, as did smaller numbers of other fish species. In August, 1997, there were several fish kills in the Pocomoke. *Pfiesteria piscicida* and at least one other toxic *Pfiesteria*-like dinoflagellate species were seen in light and scanning electron microscope analysis of water samples from the affected rivers (personal communication, J Burkholder, North Carolina State University, USA). Species in the toxic *Pfiesteria* complex had been identified as the cause of similar lesions and massive fish kills in North Carolina,[1–3] with laboratory studies showing that the neurological symptoms and skin lesions in fish were associated with release of specific toxins into the water.

Watermen working on the Pocomoke estuary began to experience health difficulties in autumn, 1996. Symptoms became more prominent during fish epidemics in spring, 1997, when reports of human illness first reached the county health department. Complaints included fatigue, headache, respiratory



Chesapeake Bay and affected waterways (detail of affected area, right)

irritation, diarrhoea, weight loss, skin irritation and rashes, and memory difficulties. With concerns accentuated by fish kills, the Maryland Department of Health and Mental Hygiene asked the University of Maryland School of Medicine and the Johns Hopkins University School of Medicine in August, 1997, to assess these complaints further.

Methods

We assessed 24 people who had had direct contact with the Pocomoke River and other estuarine waters of the Chesapeake Bay during periods of fish kills or at times when fish with active *Pfiesteria*-like lesions were present. This group included seven of the eight watermen who regularly harvested fish or shellfish on the Pocomoke River. Individuals were usually assessed within 2 weeks of their last exposure to affected waterways, with many still having daily water contact at the time of their initial examination. Studies were approved by the appropriate institutional review boards at the University of Maryland, Baltimore, the Johns Hopkins University, and the Maryland Department of Health and Mental Hygiene.

Exposure categories

We divided cases into high, medium, and low exposure comparison groups based on the following criteria:

High exposure—People in contact with affected waterways for 6–8 h per day on an almost daily basis. This category included extensive contact of skin with water, as well as exposure to aerosolised spray from the water.

Moderate exposure—People who spent 8–20 h per week assessing fish or collecting water samples from affected waterways for periods ranging from several weeks to several months. In some instances, this category included intense exposure to affected waters during fish-kill events. Most individuals wore some type of protective clothing (rubber gloves, boots, aprons) but did have contact with affected fish and the water from which they were collected; these individuals also reported exposure to aerosolised spray.

Low exposure—Individuals who had little or no direct skin contact with water, but were exposed to the Pocomoke by riding in boats during active fish-kill events. Total exposure time was generally less than 40 h per person in total.

Study participants

Given a structured questionnaire, all exposed people provided detailed information on contact with affected waterways and with fish that had skin lesions or behaviour characteristic of exposure to toxins produced by *P piscicida* or *Pfiesteria*-like species. We also asked individuals about the presence or absence of symptoms that, in initial interviews, were purported to be linked with exposure to affected rivers.

For analysis of symptom data, people in the high-exposure group (most of whom were watermen) were compared with a group of eight control watermen recruited from the ocean side of the Delmarva peninsula, where *Pfiesteria* activity had not been recorded. 13 people in the high-exposure and moderate-exposure groups underwent detailed medical and dermatological examinations. We carried out pulmonary-function testing, skin biopsy if indicated, delayed-type hypersensitivity testing (skin tests for anergy using mumps and candida antigens), and laboratory assessments. Laboratory tests included complete blood count with differential serum electrolytes, blood urea nitrogen, creatinine, liver panels (bilirubin, albumin, and transaminases), lymphocyte subset determinations (CD3, CD4, CD8, CD19, and CD16), and quantitative immunoglobulin measurement by nephelometry (IgG, IgA, IgM).

Neuropsychological studies

All participants were screened with standard neuropsychological measures. The psychometric tests were selected on the basis of several factors, including the previously reported complaints about cognition and memory of individuals exposed in a laboratory setting to *Pfiesteria*-related toxins;[1] complaints of confusion, forgetfulness, and prospective memory difficulties that had been relayed to Maryland community physicians; knowledge that the putative deficits were potentially subtle and not detectable by standard neurological mental-status examination (D E Schmechel, Duke University Medical Center, Durham, NC, USA; August, 1996: personal communication); and the psychometric properties of the tests. We selected tests with adequate published reliability and validity, norms based on age and education, test-retest reliability, alternate and equivalent forms (when possible), and previously demonstrated sensitivity to the potential effects of neurotoxins. The test battery included four experimental measures, six measures to describe or equate the study groups,

	High exposure (n=11)	Moderate exposure (n=7)	Low exposure (n=4)	Controls (n=8)
Neuropsychological symptoms: confusion, episodes of disorientation, new or increasing forgetfulness, or difficulties concentrating	9 (82%)	6 (86%)	2 (50%)	1 (13%)
Headache	9 (82%)	5 (71%)	2 (50%)	1 (13%)*
Skin lesions	8 (73%)	4 (57%)	1 (25%)	1 (13%)†
Skin burning on contact with water	5 (45%)	6 (86%)	1 (25%)	2 (25%)‡
Diarrhoea	5 (45%)	4 (57%)	2 (50%)	1 (13%)
Nausea/vomiting	7 (64%)	4 (57%)	0	1 (13%)
Abdominal cramps	4 (45%)	4 (57%)	1 (25%)	1 (13%)
Joint pain	5 (45%)	2 (29%)	1 (25%)	2 (25%)
Muscle/leg cramps	8 (73%)	2 (29%)	1 (25%)	2 (25%)
Eye irritation	6 (55%)	2 (29%)	2 (50%)	4 (50%)
Sinusitis	5 (45%)	5 (71%)	3 (75%)	3 (32%)
Shortness of breath	2 (18%)	4 (57%)	1 (25%)	2 (25%)
Pneumonia	2 (18%)	1 (14%)	0	0

*p<0·01, Fisher's exact test, two tail, comparing high-exposure group with controls.
†p<0·05, Fisher's exact test, two tail, comparing high-exposure group with controls.
‡ Control watermen reported seeing sea nettles in association with sensation of burning skin; affected individuals denied sea nettle contact. If two controls with complaints of skin burning are presumed to have had contact with sea nettles and are excluded, p<0·05, Fisher's exact test, two tail.

Table 1: **Symptoms of people examined**

and four measures to screen for factors that could potentially confound test administration or interpretation. The four experimental measures (full details of which are available on request from the authors) were as follows:

Rey Auditory Verbal Learning Test (AVLT)—We used this test[4,5] to measure the capacity for new verbal learning and memory for complex verbal information. Reliable, alternate forms are available: List A[6] was used for the first assessment (time 1) and List C[6] for the follow-up assessment (time 2). Scores from trial 5 of the learning trials were used for primary data analysis as a measure of the individuals' maximum capacity for learning or encoding new complex verbal information. We made standard score conversions (T-score) using published norms for trial 5.[7]

Stroop Color-Word Test—We administered this test[8] to assess resistance to interference and selective attention. Scoring was completed for each trial on the basis of the number of words or colours read in 45 s; we used the T-score for the colour-word interference for data analysis.[9]

Trails B of the Trailmaking Test—This test[5,10] assessed complex, divided-attention, or multiple-tracking abilities.[5] Scoring is based on the time to accurate task completion and T-score conversions were made by means of the norms of Davies.[12] At the second administration, we used an alternate, equivalent form from the Pathways Test.[11]

Grooved-Pegboard Test—We administered this test to assess fine motor speed and dexterity. We used the time to completion for the participant's dominant hand for data analysis; T-score conversions were made by use of published norms.[13]

The measures we used to describe or equate the exposed and non-exposed participant groups included the Digit Span (simple attention and concentration), Digit Symbol (visual scanning, clerical speed and accuracy) and Block Design (constructional praxis) subsets of the revised Wechsler Adult Intelligence Scale (WAIS-R), and the Controlled Oral World Association Test (COWA; verbal fluency). The Visual Retention Test was used to assess simple visual memory; this test involves the immediate reproduction of easy geometric designs. We also calculated a Barona index for each participant as an estimate of premorbid intelligence.[14] This demography-based index gives a premorbid IQ estimate for the WAIS-R.

We administered six measures to assess potential confounding factors in the administration or interpretation of the neuropsychological tests. These included the Profile of Mood States to screen for anxiety or depression, psychological variables that could potentially impair performance on measures of memory, psychomotor speed, and selective or divided attention. Rudimentary naming and visual-perceptual abilities were also screened by means of items from the Visual Naming Test of the Multilingual Aphasia Exam and the Hooper Organisation Test. Temporal orientation (Temporal Orientation Test) and reading comprehension (selected items from the Boston Diagnostic Aphasia Exam) were screened to insure that the individual was capable of completing valid informed consent, interview, and neuropsychological test procedures. Finally, we used the Rey 15-item Memory Test as a screen for factitious or simulated memory difficulties.[4] If the participant obtained a score of 9 or lower on the test or if we had any other reason to suspect functional memory complaints (eg, symptom exaggeration beyond clinical performance, recognition memory more impaired than free-recall performance on Rey AVLT), we reviewed the participant's history and other potential indicators of functional memory complaints for corroborating evidence of the potential for feigning memory impairment.[15] If there was any reason to suspect feigned memory impairment or symptom exaggeration, the participant was excluded from the data analysis. People with functional or anatomical reasons for test abnormalities were also excluded from analysis.

Controls were selected for exposed people on the basis of age, sex, and educational and occupational status. All tests were administered and scored according to standard procedures by trained examiners unaware of the exposure histories of the exposed and 11 of 19 control participants. We used scaled or standardised T-scores for data analysis at the time of initial testing to allow a more direct comparison across participants and measures. Raw scores were used for change-score calculations computed after follow-up assessment.

Statistical methods

We used parametric analyses to assess the differences in T-scores and changes in raw scores among the four exposure groups (the within-group frequency distributions were not

	Exposure				Total
	None	Mild	Moderate	Severe	
Mean (SD) age in years	38·94 (8·93)	38·00 (4·36)	39·43 (12·41)	39·89 (10·36)	39·18 (9·53)
Mean (SD) education in years	13·32 (2·49)	13·67 (2·08)	14·71 (2·36)	11·22 (2·05)	13·32 (2·49)
Sex (M/F)	15/4	3/0	4/3	8/1	30/8

Table 2: **Demographic variables**

	Exposure				Results	
	None (n=19)	Mild (n=3)	Moderate (n=7)	Severe (n=9)	ANOVA	Linear trend
Mean (SD) Rey AVLT trial 5 T-score	54·21 (7·32)	35·33 (14·74)	38·57 (13·15)	19·56 (16·69)	p<0·0001	p<0·0001
Mean (SD) Stroop Interference T-score	51·58 (7·99)	39·00 (2·00)	48·83 (11·36)	39·78 (10·87)	p=0·011	p=0·006
Mean (SD) Trails B T-score	57·32 (7·59)	48·67 (7·77)	50·71 (14·27)	48·11 (10·87)	p=0·100	p=0·021
Mean (SD) Grooved Pegboard T-score	52·53 (9·87)	43·33 (14·43)	54·29 (11·70)	36·11 (14·09)	p=0·006	p=0·008

Table 3: **Experimental neuropsychological measures at time 1**

substantially skewed and tests for heterogeneity of variances were not significant). A one-way ANOVA was used to assess the overall between-group variability in means. We tested for a dose-response trend using the linear component of the between-group sum of squares. We used a Student's *t* test to measure significance for the comparison of mean T-scores and mean changes between exposed watermen and their own control group.

Results

Two of the 24 exposed people were excluded from analysis because complete data on symptoms were not available. The most commonly reported symptoms that distinguished the exposed from the control groups were neuropsychological, including acute confusion, episodes of disorientation, new or increasing forgetfulness, or difficulties concentrating (table 1). Memory difficulties described by patients included driving in a car towards predetermined destinations, unable to recall where they were expected to go or what they should do on arrival. They described doing such activities as mailing packages, then forgetting that they had done so. Those who needed to remember measurements were unable to remember numbers; others could not remember to bring routine equipment on board boats, and found themselves on the water without required supplies.

Other reported symptoms included headaches, skin lesions, and skin burning on contact with water. Skin burning was consistently described as an episodic sensation ("like acid" or "stinging") that was relieved by washing with bottled water or resolved spontaneously within 12 h after water contact. Although not significantly different from controls, exposed individuals also reported severe leg cramps and acute respiratory difficulties or irritation associated with exposure to affected waterways. No consistent or unexpected abnormalities were identified on physical examination. Pulmonary function was normal or consistent with history of tobacco use. Laboratory studies were normal except for one person who had a urinary-tract infection. Exposed people showed normal immunological function with appropriate responses to antigens, normal T-cell subset percentages and absolute counts, and normal immunoglobulin concentrations. Complete blood counts and differentials, serum electrolytes and creatinine, transaminase concentrations (alanine aminotransferase and asparate aminotranferase), and albumin and bilirubin concentrations were normal in all but one person (with raised alanine aminotransferase and asparate aminotransferase due to what seemed to be a previously unrecognised infection with hepatitis C virus).

Full cutaneous examinations revealed four exposed people with unexplained or suspicious lesions; three lesions were erythematous papules (arm, abdomen, and flank) and one was a scaling patch on the foot. All lesions appeared non-descript; the papules resembled arthropod bites, and the patches resembled nummular eczematous dermatitis. In addition, several common dermatoses occurred, including lamellar dyshidrosis, pruritic, or vesiculated skin lesions, which resolved. Review of clinical photographs of one person taken at the time of initial onset of symptoms revealed several annular erythematous patches and plaques over exposed body surfaces.

The most common findings by skin biopsy included a superficial and/or deep perivascular, periadnexal, or interstitial lymphohistiocystic infiltrate (six of eight biopsy samples assessed), epidermal spongiosis (four of eight), and prominent eosinophils (two of eight). Periodic-Acid/Schiff and Brown and Bren Stains were negative in five of five specimens tested. We recorded no dinoflagellate forms. Findings were consistent with reactive erythema or allergic or toxic reaction (four of eight), folliculitis versus toxic reaction (two of eight), eczematous versus psoriasiform dermatitis (one of eight), and inflamed verrucous keratosis (one of eight).

Neuropsychological studies

General tests—Of the 24 exposed people seen for neuropsychological examination, five were excluded from data analysis because factors other than *Pfiesteria* exposure could potentially explain their abnormal findings (previous right-cerebral-hemisphere tumour resection [one]; developmental learning disabilities

	Exposure				Results	
	None (n=12)	Mild (n=1)	Moderate (n=4)	Severe (n=5)	ANOVA	Linear trend
Mean (SD) Rey AVLT trial 5 change score	0·75 (1·66)	3·00 (0)	2·25 (2·06)	4·20 (1·30)	p<0·001	p<0·001
Mean (SD) Stroop Interference change score	0·00 (6·80)	−2·00 (0)	3·00 (6·83)	12·60 (5·68)	p=0·017	p=0·004
Mean (SD) Trails B change score	−4·63 (10·36)	−21·00 (0)	−4·75 (8·85)	−29·60 (35·1)	p=0·099	p=0·057
Mean (SD) Grooved Pegboard change score	3·82 (7·40)	−2·00 (0)	−2·00 (4·55)	−1·60 (12·54)	p=0·010	p=0·010

Table 4: **Change scores on experimental neuropsychological measures**

	Exposure		ANOVA
	No (n=12)	Yes (n=10)	
Mean (SD) Rey AVLT trial 5 T-score	51·75 (10·09)	40·90 (11·36)	p=0·059
Mean (SD) Stroop Interference T-score	51·75 (7·34)	53·20 (6·32)	p=0·405
Mean (SD) Trails B T-score	56·50 (8·30)	58·80 (5·03)	p=0·558
Mean (SD) Grooved Pegboard T-score	44·33 (16·53)	48·30 (9·90)	p=0·513

Table 5: Experimental neuropsychological measures at time 2

[two]; suspected exaggerated memory impairment [one]; and a premorbid history of abnormal mental decline suggestive of multi-infarct dementia [one]). The 19 remaining exposed individuals were matched with 19 controls (non-exposed participants) based on age, sex, and educational and occupational status (table 2). Both the exposed and non-exposed groups each comprised eight commercial fishermen, one skilled labourer, five semi-professional employees, three clerical or technical employees, and two graduate students as members.

Based on screening examination, all participants were temporally oriented and there was no difference between exposure groups with respect to these scores (t=0·30, p=0·35). All participants also showed intact and equivalent performances on screening measures of reading comprehension (all had 100% accuracy), perceptual judgement (t=1·51, p=0·14), and naming (t=1·02, p=0·31). The average Barona index for premorbid full-scale IQ was 104·5 for the exposed individuals and 105·1 for the non-exposed participants (t=0·23, p=0·81). The estimated premorbid verbal IQ was 104·8 for the exposed individuals and 105·9 for the non-exposed people (t=0·41, p=0·68). The estimated premorbid performance IQ for the exposed individuals was 102·7, and 103·4 for the non-exposed participants (t=0·30, p=0·76).

Similarly, we found no significant differences between the exposed (mean 9·89) and non-exposed (9·68) groups with respect to performance on measures of simple attention (Digit Span, t=0·26, p=0·80); constructional praxis (Block Design, exposed mean 10·78, non-exposed mean 10·84; t=0·07, p=0·94); verbal fluency (COWA, exposed mean 39·59, non-exposed mean 40·16; p=0·86); or simple visual memory (Visual Retention Test errors, exposed mean 4·72, non-exposed mean 3·95). On the Digit Symbol subtest of the WAIS-R, which measures clerical speed and accuracy, we found a significant difference between the groups—the exposed individuals obtained a mean score of 9·83 (SD 2·01) compared with the non-exposed-group mean of 11·95 (2·72; t=2·67, p=0·01). Follow-up analysis of this data indicates that this difference is accounted for by the severely exposed group (mean 8·63) compared with the entire non-exposed group, and is not maintained when the watermen are compared with their own occupational controls (exposed-watermen mean 8·67 [SD 2·34], non-exposed watermen mean 11·13 [3·13; t=1·54, p=0·15]).

None of the exposed or non-exposed individuals obtained scores outside the normal range (a T-score >60 or <40) on the depression or anxiety scales of the Profile of Mood States. Two severely exposed watermen obtained T-scores of 66 on fatigue subscale, endorsing items consistent with complaints of feeling tired. Six of eight exposed watermen and seven of eight control watermen had increases above the normative mean on the Vigor Scale—a subjective measure of physical robustness, energy, enthusiasm, and well-being.

Finally, we found no difference in performance on the Ray 15-item measure (simulated memory screen) between the two groups—the exposed participants obtained a mean score of 14·5 and the non-exposed individuals a mean score of 14·8 (t=0·87, p=0·38).

Experimental measures—There was a significant difference between the four exposure groups with respect to performance on Trial 5 of the Rey AVLT, the Stroop Color-Word Test, and the Grooved Pegboard Test (table 3). There was no significant difference for the exposure groups with respect to performance on the Trails B test. We also calculated a significant linear trend for the AVLT score, Stroop Color-Word Test, and Grooved Pegboard task in the direction of worse performances associated with more severe exposure.

Four other measures of the Rey AVLT were analysed, including the Trial 1 Learning score, the score for total number of words learned over five trials, the delayed-recall score, and the recognition score. There was no difference between exposed individuals and controls for the number of words learned at trial 1 (t=1·15, p<0·26). We found significant differences between the cases and the controls for the total number of words learned over all five learning trials (t=4·80, p<0·001). The high-exposure group learned significantly fewer words than the moderate, low, or control groups (F=11·3, p<0·0001), with a significant linear trend (p<0·0001). We also found significant differences between the groups in the delayed free-recall and recognition scores (F=9·34, p<0·0001 *vs* F=5·51, p=0·004). However, these latter differences were not significant after controlling for number of words learned.

Most of the individuals in the severe-exposure group (eight of nine) were watermen, an occupational group with the least education and a unique range of occupational experiences and exposures. Hence, in separate analysis, we compared the findings of this group with their matched occupational controls. Findings indicate that a significant difference in performance was maintained between the exposed and the non-exposed watermen (mean 14·57 [SD 7·00] vs 55·50 [5·42]) on Trial 5 of the AVLT (p<0·0001). Differences also occurred between the exposed and the non-exposed watermen (38·14 [9·97] *vs* 50·88 [5·82]) on the Stroop Interference Task (p=0·009). Finally, we calculated a significant difference in performance on the Grooved Pegboard task: the exposed watermen obtained a mean T-score of 36·43 (SD 13·76) compared with 50·63 (11·78) for the non-exposed watermen (p=0·05).

When data from the excluded cases were included in these analyses, they strengthened the observations. We also compared the findings of people with exposure histories known to the examiners and those for whom the examiners were unaware of exposure status. There were no differences in scores on any cognitive measure for these two groups.

Neuropsychological follow-up of exposed cases—Within 14 days of our initial examination, estuarine waters known to have fish with lesions and behaviour

suggestive of toxins produced by *Pfiesteria piscicida* or *Pfiesteria*-like species were closed to the public. To our knowledge, the exposed individuals had no further exposure to the affected waterways.

10–12 weeks after the most recent exposure, follow-up studies were undertaken to assess the natural time-course of recovery from exposure. All the previously exposed individuals were invited for follow-up testing. 14 returned for testing. Two of 14 had been excluded from initial analysis, and were excluded again from analysis on repeat testing. For two additional people, there were uncertainties about possible symptom exaggeration on retesting. The remaining ten participants met the inclusion criteria described in study 1, were included in study 1, and were subsequently included in the data analysis for study 2. The exposed individuals lost to attrition (n=5) did not differ with respect to demographic or neuropsychological measures at time 1 from those who returned for testing. 12 of the original non-exposed individuals, matched for age, education, sex, and occupational status, also underwent repeat testing. All participants for the follow-up testing received the same battery of tests administered at time 1; alternate forms were used when available.

ANOVA of change scores indicated a significant improvement in scores on trial 5 of the AVLT and the Stroop Interference Test (table 4). The exposed groups learned significantly more words on the list by trial 5 and were less susceptible to interference on the Stroop. There was also a linear trend in these improved performances, with those individuals who had the worst initial performance showing the most improvement in follow-up assessment. Once again, because the watermen from the most severely exposed group are a unique occupational group, we compared their data with their own non-exposed occupational controls. The findings were consistent with the larger sample analysis, since the change score of the exposed watermen was significantly greater (in the direction of improvement) than that of the non-exposed watermen on the AVLT trial 5 score (mean 4·20 [SD 1·30] *vs* −1·8 [1·79]). The possibility of ceiling effects for the control group arose by use of trial 5 of the AVLT as a learning score. Hence, we did additional data analyses using the total number of words learned across all five trials as a measure of new learning. Our findings were strengthened when total number of words learned was used for data analysis. The mean improvement on the Stroop Interference score for the exposed individuals was 12·60 (SD 5·68) compared with 1·80 (5·36) for the non-exposed controls (p=0·015).

When the scores of exposed and non-exposed people were compared at follow-up (table 5), there was no significant difference on the Grooved Pegboard task, the Stroop Interference score, or the Trails B test. A significant difference remained on the trial 5 score of the Rey AVLT, despite significant improvement among those in the exposed groups. On further analysis of these data, two of the most severely exposed and affected individuals continued to have deficient or impaired performance on this measure, despite improvement from the first testing period. The other exposed participants had improved to well within the mean performance range.

The two people with residual impairments at their 10–12 week follow-up examination were studied again at 6 months' post-exposure. They reported improvement in their functional daily memory, and had normal performances on all cognitive measures.

Discussion

We report a new clinical syndrome that includes alterations in specific aspects of neuropsychological function. Complaints of memory difficulties, headache, skin lesions, and skin burning upon contact with water dominate the clinical presentation. The most consistent objective finding among exposed individuals is a pattern of deficits in new learning and selective and divided attention. These deficits occur within the context of otherwise normal performances on other neuropsychological measures—including tests of temporal orientation, constructional praxis, visual memory for simple geometric designs, attention, concentration, and language abilities—and cannot be attributed to age, education, occupational status, or other premorbid neurological, psychiatric, or academic factors. On the basis of our repeat-testing results, deficits may be expected to improve significantly within 3–6 months after cessation of exposure to affected waters.

Our sample of patients, though largely self-selected, encompassed most of the people known to have moderate-to-high exposure to the Pocomoke River. Our data thus suggest a picture of the more severe end of the spectrum of human-health effects associated with chronic exposure to water when toxin-producing *Pfiesteria piscicida* and other toxic *Pfiesteria*-like species are present. Milder manifestations might exist, which may not be readily detectable on routine screening examination. At the other end of the spectrum, there are reports of a global confusional syndrome associated with acute exposure to the toxin;[1] further studies are needed to confirm and characterise these symptoms. Some cutaneous lesions were found that could not be otherwise explained. Although clinical and histopathological findings were consistent with a toxic allergic reaction, further studies are needed to determine their significance.[16] We did not see evidence of respiratory difficulties that could be directly linked to exposure to affected waterways. Again, however, this symptom may be more transient than the neuropsychological manifestations, and would be more prominent in a cohort of acutely exposed individuals.

Identification of the syndrome is also complicated by our current need to rely on neuropsychological criteria for its diagnosis. From the beginning, we were concerned about the possibility of symptom exaggeration or malingering among participants,[5] and took great care in designing the test battery to enable us to identify such behaviour.[15,17] In our initial testing, there was only one instance in which we felt there might be symptom exaggeration. Participants were unfamiliar with the test measures; neither we nor the participants knew which, if any, results might prove important or significant in defining the syndrome. Also, participants tended to be young, vigorous, self-employed individuals who had little to gain by feigning illness. In our repeat testing (at a time when there was local public awareness of a *Pfiesteria*-associated syndrome, information about critical tests was more widely known, and people had experience with the test format) we saw more evidence

of possible symptom exaggeration, leading us to exclude two additional individuals from the follow-up analysis. We also recognise that inherent in the use of multiple neuropsychological screening measures is a risk of inappropriate focusing on the statistical significance of a single test. However, the overall pattern of neuropsychological disturbance seen in our patients, combined with the extent to which these measures systematically fluctuated with exposure level and recovery, argues against this possibility.

The patients examined in this study expressed their cognitive impairment in terms of memory difficulties. However, neuropsychological testing identified what seemed to be a higher cognitive dysfunction that could interfere with functional or everyday memory. The most striking impairments were on the learning measures of the Rey AVLT, which involves recall of a list of 15 unrelated words for five trials; 30 min after the last trial, the person is asked to recall as many words from the list as they can in both free-recall and recognition paradigms. To learn this list of 15 unrelated words successfully, an individual must be able to allocate complex attentional, divided-attention, and executive resources towards forming new associations. In our patients, disruption of selective and divided-attention capacities may have selectively limited their capacity for learning lists of complex words but not for delayed recall (ie, they had difficulties in learning, but, once something was learned, they could remember it). This hypothesis is consistent with a 1996 study that suggested that divided-attention deficiencies selectively impair the encoding or initial learning process of memory, but not retrieval of the newly learned information.[18] This pattern of neuropsychological findings has not, to our knowledge, been reported in association with exposure to other dinoflagellate toxins.[19–23] Interestingly, there are suggestions that similar impairments in higher cognitive function (in a very different clinical setting) underlie some of the memory disturbances seen in people with exposures to organic solvents.[24,25]

To date, the dinoflagellate species and toxins that lead to this syndrome have yet to be fully characterised. Hence, we cannot say definitively that the clinical syndrome we recorded is directly caused by toxins produced by *Pfiesteria piscicida* or *Pfiesteria*-like dinoflagellates. However, a convergence of evidence supports this hypothesis. First, onset of symptoms in our cases occurred during periods when erratic fish behaviour and numerous fish with lesions were seen, with no other clear reason found to explain the human presentations. Second, *Pfiesteria piscicida* and a second toxic *Pfiesteria*-like species were identified in the Pocomoke estuary in May during a fish-lesion epizootic and again at the time of the August fish kills; also, fish in the affected waterways had the very distinctive lesions known to be associated with fish exposed to *Pfiesteria* toxins in natural and laboratory settings.[3] Third, the reported cognitive deficits in three people exposed to *Pfiesteria*-like dinoflagellates and *Pfiesteria* toxins in the laboratory were similar to those of our cases.[1] Fourth, there is now a report[26] of learning difficulties in laboratory rats associated with exposure to water from aquaria containing *Pfiesteria* toxins. Finally, we observed a signficant dose-response effect. Individuals with more intense exposures had more severe disease; these individuals also showed a significantly greater degree of recovery after cessation of exposure to affected waterways.

Our investigation of the human-health effects of exposure to waterways containing toxic *Pfiesteria* or *Pfiesteria*-like dinoflagellates has allowed us to outline elements of a clinical syndrome and generate potentially helpful hypotheses for further study. The link between *Pfiesteria* and *Pfiesteria*-like dinoflagellates, their toxins, and health effects in humans beings, needs to be clarified. Mechanisms responsible for the observed neuropsychological symptoms remain to be defined. Data are needed on the type and degree of exposure necessary to cause clinical symptoms, and further information on the natural history of illness is required. More comprehensive neuropsychological measures are needed to characterise further the potential range of neurocognitive symptoms. More fundamentally, there is a need to explore the environmental basis for the emergence of this new pathogen (with its propensity for killing fish) in the Chesapeake Bay region, and its relation to toxic *Pfiesteria*-like dinoflagellates in other coastal areas.

Contributors

Lynn M Grattan was responsible for selection and administration of the neuropsychological tests and the writing of the paper. David Oldach had a key role in the assessment of patients and in the study design. Trish M Perl participated in epidemiological design of the study and in case identification. Mark H Lowitt was responsible for design and implementation of dermatological aspects of the study; he was assisted by Lisa Kauffman. Diane Matuszak and Martin P Wasserman were responsible for setting up the initial case assessments, and for outlining the scope of the studies. Curtis Dickson and Colleen Parrott are with the county health department, where the studies were done, and were responsible for designing and recruiting the panels of patients. Ritchie C Shoemaker first assessed patients with *Pfiesteria* exposure, for whom he contributed data to the paper. J Richard Hebel assisted in study design and carried out statistical analyses. Patricia Charache participated in the medical assessment of the study participants, and was the lead Johns Hopkins physician on the team. J Glenn Morris was team leader, with contributions to the design and implementation of the study, and the writing of the paper. All authors reviewed and contributed to the preparation of the final typescript.

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