Potential Human Health Effects Associated with Laboratory Exposures to *Pfiesteria piscicida*

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The adverse human health effects associated with the most prolonged and intense exposure known to *Pfiesteria piscicida* Steidinger & Burkholder cultures and toxin(s) are described. In December 1993, a patient presented with acute illness to the Memory Disorders Clinic of the Bryan Alzheimer's Disease Research Center at Duke University Medical Center with significant cognitive deficits 2 weeks after ceasing occupational laboratory exposure on the recommendation of the evaluating primary care physician. The clinical and exposure histories of this patient are presented. The comprehensive neurological examination findings are reviewed, with attention to the patient's neuropsychological evaluation. Six-week follow-up data illustrate the course of symptom resolution with exposure cessation. This case is presented in an effort to contribute to the gradually accruing evidence of potential central nervous system sequelae of *Pfiesteria* exposure. The case is discussed in the context of additional cases evaluated at Duke University Medical Center and the complicated scientific framework in which such evaluations proceed while definitive surrogate or biological markers are awaited. *Key words*: human health, laboratory exposure, *Pfiesteria piscicida*. — *Environ Health Perspect* 109(suppl 5):775–779 (2001).
http://ehpnet1.niehs.nih.gov/docs/2001/suppl-5/775-779schmechel/abstract.html

Pfiesteria piscicida Steidinger & Burkholder is a relatively newly recognized species of toxic estuarine dinoflagellate inhabiting east coast waterways in the United States. This organism has been associated with numerous large fish kills in these areas since its discovery in 1991 (*1*). *Pfiesteria* has a complicated life cycle involving approximately two dozen morphologically distinct life forms. Although it is thought that at least two toxins are released at certain stages of the life cycle, as yet they have not been purified and characterized fully. These toxins result in stunning of fish prey and are associated with rapid development of skin lesions on the fish. However, as yet there is no specific or even surrogate biomarker for exposure.

In humans, known occupational and putative civilian exposures to *Pfiesteria* have been associated with memory complaints, confusion, skin burning or rash, headaches, eye irritation, respiratory irritation, muscle cramps, and gastrointestinal distress (*2*). Formal investigations have established an association between exposure to *Pfiesteria* and deficits in information processing. Specifically, learning and higher cognitive function deficits were related to contact with waterways containing fish exposed to toxins produced by *Pfiesteria* or *Pfiesteria*-like dinoflagellate species on the Pocomoke River and adjacent waterways in Maryland (*3*). Severity of illness was related to the degree of exposure, and test scores returned to within the normal range after a period of exposure cessation (*3*). The observed resolution of symptoms was consistent with that suggested in reports of some cases followed longitudinally at Duke University Medical Center in Durham, North Carolina (*2*). The potentially transient nature of symptoms associated with the syndrome is also indirectly supported by the lack of evidence for marked, consistent deficits in a North Carolina cohort studied 2–3 months after the last recognized fish kill involving *Pfiesteria* (*4*). Finally, learning and processing difficulties in rats exposed to extracts of the *Pfiesteria* toxin have also been reported (*5,6*), and these effects were also thought to be dose responsive. Overall, the present state of knowledge suggests that exposure to *P. piscicida* can result in a mild, transient encephalopathy primarily affecting learning and memory and higher-order executive functions. However, data on the potential central nervous system (CNS) effects of exposure is sparse, and large epidemiological cohort studies currently under way have been hindered by the recent drop in *Pfiesteria*-related fish kills and the lack of a biomarker to assist in case identification.

The index case of prolonged, intense laboratory exposure to *P. piscicida* presented to the Memory Disorders Clinic (MDC) of the Bryan Alzheimer's Disease Research Center (ADRC) at Duke University Medical Center in December 1993. This patient has been followed since that time and is now presented in an effort to delineate some of the potential adverse human health effects associated with exposure. To protect confidentiality, we removed all identifying information. The case is then discussed in the context of other laboratory and civilian cases that have presented to the MDC for evaluation. Finally, the theoretical and scientific frameworks in which *Pfiesteria* exposure sequelae research progresses is considered.

Case History

The patient was a healthy, well-educated Caucasian with no known risk factors for illness. Medical history was negative for known or suspected illnesses affecting the CNS. Family history was also negative for neurological and psychiatric disorders.

Exposure History

During 1991 and 1992, the patient had gradually increasing research responsibilities involving cultures of toxic forms of *P. piscicida*. These activities involved feeding, cleaning, and harvesting in an enclosed room housing aquaria with fish and *Pfiesteria* cultures. Exposures were inhalational and dermal in the humid atmosphere of this room and included handling of killed and decomposing fish resulting from cultures of the activated forms of *Pfiesteria*. During this time, the patient reportedly experienced several distinct episodes of euphoria and disorientation. Interestingly, there was a relative inability to terminate exposure despite realization of these symptoms and a sense of urgency to finish tasks. The patient experienced relatively rapid recovery within an hour after these events and was able to function adequately between episodes without major adverse effects. During 1993 additional safety precautions including gloves and respirator masks were

taken because of increased culturing activity. However, an unfortunate construction error during expansion of the laboratory physical layout resulted in cross-ventilation of the aquarium heating, ventilation, and air-conditioning to the desk area of the patient. Exposure during this 8- to 9-month period was reported to be daily for 8–10 hr. In addition to aerosol exposure, dermal exposures around the patient's glove area reportedly resulted in skin sores. In late 1993, the patient experienced a rapid 1- to 2-month deterioration, with symptoms consistent with what is termed descriptively an organic-affective syndrome or toxic encephalopathy. Mood lability, poor work performance, trouble reading and doing scientific work, disorientation while driving, and dermal sores on his extremities led to realization by family and colleagues that he was medically ill. The patient had reduced insight, but the severity of symptoms led to medical examination of the patient by his primary care physician and a recommendation for immediate cessation of exposure and medical leave from work. Screening laboratory tests were normal except for mildly elevated liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT)] less than 2 times the upper normal range. Signs and symptoms began to improve rapidly in the days following cessation of exposure.

Initial Examination

The patient was referred for neurological evaluation in December 1993, roughly 2 weeks after exposure cessation and while still recovering. The following section outlines findings from the comprehensive physical and neurological examinations completed at that time.

Subjective Complaints

The patient presented with numerous complaints, many of which suggested changes in neurocognitive abilities. The reported symptoms included recent memory impairment, declined word-finding and reading skills, spatial disorientation, rage and emotional lability, headaches, skin sores and poorly healing infections, and autonomic symptoms involving perceived and measured changes in pulse, blood pressure, and temperature (hyperautonomic).

Physical Exam

The patient was alert and cooperative and had normal vital signs. General physical examination was normal except for some healing 2- to 4-week-old skin sores roughly 2–4 mm in size on the wrist and dorsum of the hand, with the appearance of excoriated eczematous dermatitis. Slight hyperhidrosis was present in the palms.

Neurological Exam

The patient's neurological examination was mostly normal. Gait, cranial nerves, motor function, coordination, sensory function, and reflexes were all within normal limits. However, observations of mental status were notable for hesitant speech, decreased fluency, and psychomotor slowing.

Because of these neurological findings, the patient was referred for comprehensive neuropsychological evaluation and urgent laboratory studies. Because of adequate functioning and observed improvement and no indication of the nature of the putative toxins, the patient was not hospitalized and no medical intervention was taken.

Laboratory and medical findings. Bloodwork revealed normal screening labs, including complete blood count and routine chemistries, with the exception of low serum phosphorus and slightly elevated liver function (AST, ALT) tests, which resolved by the second visit 1 week later. Other ancillary tests were also well within normal limits, including urinalysis, $TmPO_4$ (renal threshold phosphate excretion) for renal threshold for phosphate excretion, and plasma and urine amino acids. Skin biopsy revealed nonspecific changes with microhemorrhage and infiltrate without marked eosinophilia, consistent with secondary infection and eczematous dermatitis.

Neurophysiological findings. Electromyography-nerve conduction studies were normal, as were visual and brainstem evoked potential studies. Sleep-deprived electroencephalography was also entirely within normal limits.

Neuroimaging findings. Magnetic resonance imaging studies revealed a slight asymmetry of the hippocampi, with the left hippocampus having minimally increased T2 signal intensity compared to right. In contrast, fluorodeoxyglucose positron emissions tomography scan findings were normal without any cortical or subcortical areas of increased or decreased glucose utilization in the basal state.

Neuropsychological Evaluation

Clinical neuropsychology allows for the estimation of CNS integrity through the measurement of cognitive skills. Central to its practice is the administration of standardized, objective tests. Healthy normative group data allow for control of variables known to influence cognition, such as age and education. A comprehensive clinical examination involves assessment of multiple cognitive domains, including intellect, higher-order executive skills such as reasoning and cognitive flexibility, learning and memory, language, visual-spatial skills, fine motor skills, and mood. The resulting scores are then examined relative to standardization group norms. The pattern of performance is also considered in light of the individual's estimated premorbid levels of functioning based on demographics and scores that are less susceptible to decline, even in the context of neurological illness. The patient's raw scores were converted to standard scores, with a mean of 100 and standard deviation of 15. Figure 1 illustrates select scores representative of performance in each neurocognitive domain. For this individual, premorbid level of functioning was estimated to be well above average. Whereas for an average individual a score of 70 (two standard deviations below the mean) would indicate impairment, for a person previously estimated to have above-average and superior skills, an average score of 100 could be clinically relevant and arouses concern for decreased function. The results revealed a constellation of findings indicating a decline in functioning in specific areas.
- Higher-order executive abilities (blue bars: Trail Making Test, Wisconsin Card Sorting Test, Arithmetic subtest – Boston
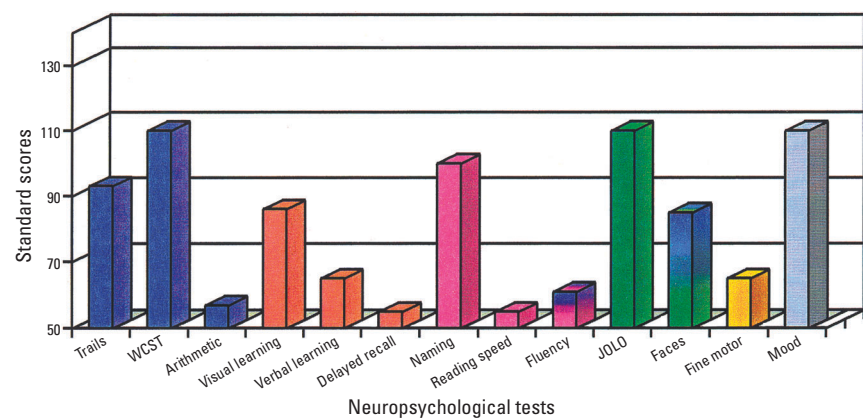

Figure 1. Initial neuropsychological evaluation test scores. JOLO, Benton Judgment of Line Orientation Test; WCST, Wisconsin Card Sorting Test.

Diagnostic Aphasia Examination were highly variable. While performance on a test of concept formation requiring the patient to attain, maintain, and shift problem-solving strategies in response to feedback was within normal limits, performance on tasks involving sequencing and cognitive flexibility suggested decreased efficiency, and arithmetic skills were markedly impaired.

- Learning and memory skills (red bars: Wechsler Memory Scale-Revised Visual Learning, Verbal Learning, and Delayed Memory Indices) were profoundly impaired. Verbal learning was disproportionately affected relative to nonverbal learning skills. Delayed recall skills indicated minimal retention of information over time, with scores suggesting a memory deficit of amnestic proportions (i.e., below the first percentile relative to age-matched peers).
- Although language abilities (pink bars: Abbreviated Boston Naming Test, Chapman-Cook Speed of Reading Test, Controlled Oral Word Association Test–Multilingual Aphasia Examination) were variable, a marked reading deficit was evident. In addition, verbal fluency was also below expectation, although this performance may have been related to executive dysfunction.
- Visual–spatial skills (green bars; Benton Judgment of Line Orientation Test, Benton Facial Recognition Test) also were variable, again with skills more closely related to executive abilities being affected to a greater degree.
- Fine motor (orange bar: Finger Tapping Test) skills were also impaired.
- Although emotional distress was reported, objective findings suggested that the patient's affective status (light blue bar: Minnesota Multiphasic Personality Inventory) did not account for the neurocognitive findings at the time of evaluation.

Initial Diagnostic Impression

The initial neuropsychological examination confirmed and documented the clinical impression of an organic amnestic syndrome. Findings were notable for a memory deficit of amnestic proportions, impaired auditory attention, slowed calculation skills, and a mild alexia. This constellation of findings conformed to what is often observed in illnesses involving frontal–temporal structures and related systems, and in this particular case the pattern of deficits suggested possible disproportionate involvement of the left hemisphere.

In addition to clear evidence of cognitive dysfunction, multisystem involvement was suspected on the basis of the history of skin lesions, as well as possible renal, liver, and autonomic nervous system findings on the basis of history and laboratory tests. Differential diagnostic considerations favored the possibility of occupational laboratory exposure to *P. piscicida* cultures as the environmental cause of the syndrome given the following: absence of other explanatory conditions, temporal relation between increased exposures and symptom onset, temporal relation between exposure cessation and symptom resolution, and parallel between degree of exposure and symptom magnitude. Excluded by the clinical course and laboratory findings were alternative diagnoses such as ischemic injury, epilepsy or seizure disorder, transient global amnesia, demyelinating disease, other toxic encephalopathy, infection, systemic metabolic disease, and indirect CNS effects of pancreatic, hepatic, or renal injury. The overall presentation suggested a cumulative effect of chronic exposures with acute decompensation throughout the fall of 1993 during increased exposure intensity. Although clinical encephalopathy was clearly present, the case did not permit an understanding of whether this represented direct or indirect effects of systemic exposure or any specific understanding of the nature of putative toxin(s). Excitotoxic mechanisms were considered (such as domoic acidlike agents), as

that would have clinical implications, but were not supported by the clinical course and laboratory findings. The exposures (primarily dermal and inhalational) suggested a lipophilic or amphiphilic agent with possible long-term accumulation in body-fat stores, lipid-rich organs (e.g., brain) or other viscera. Unfortunately, no frozen fat or other tissue biopsies were taken except for a serum sample kept frozen.

Longitudinal Course

Diagnostic Impression at Six-Week Follow-up

All contact with *Pfiesteria* was terminated with acute illness. Although all laboratory contact with toxic *Pfiesteria* was avoided, the patient remained in the aquatic biology field. The results of comprehensive reevaluation after 6 weeks suggested resolution of previously observed deficits, with mild neurocognitive residuals. Figure 2 is a comparison of follow-up and initial findings on select scores. Although practice effects could contribute to the observed improvement, they did not account for them. The magnitude of gains was such that marked recovery was indicated.

Potential Long-Term Sequelae

The patient was instructed to exclude further exposures to *Pfiesteria* and resumed work in the research laboratory. Mild symptoms reported in the months following exposure were fatigue with exercise (previously well-conditioned individual) and occasions of slight disorientation when painting using latex paints at home. Although premorbid tests were not available, the patient self-reported being an excellent reader (>1,000 words/min) prior to exposure. Thus, the improved reading score at follow-up likely reflected a continued deficit relative to estimated premorbid levels of functioning. Nonetheless, the patient returned to full, successful, and gainful employment as a research scientist.

In 1999, 6 years after exposure, the patient experienced a subacute episode of visual disturbance and pain of the right eye that resulted in inpatient admission to the Neurology Service at Duke University Medical Center. The age of the patient and nature of the presentation suggested demyelinating disease. Examination revealed a significant scotoma with essentially right temporal hemianopsia, without other neurological abnormalities. Visual evoked potential study demonstrated abnormal conduction times over the anterior visual pathway on the right, but normal brainstem and somatosensory evoked potentials. Cerebrospinal fluid (CSF) studies, contrast and noncontrasted MRI, and neuropsychological evaluation were all within normal limits. In particular, there was no
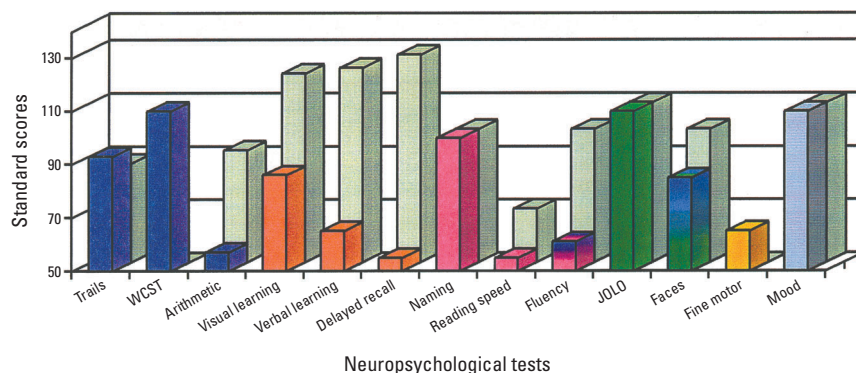

Figure 2. Initial and 6-week follow-up neuropsychological test scores.

imaging correlate to the lesion in the right anterior (prechiasmatic) optic pathway or elsewhere to support multiple demyelinating lesions. CSF studies were negative for oligoclonal bands and revealed a normal IgG index. Thus, a diagnosis of multiple sclerosis was not supported. Steroid treatment was initiated, with resolution of many of the symptoms, and a second course was performed a few weeks later for a brief recurrence of symptoms. Significant visual deficit remains in the right eye, but there have been no further neurological episodes.

The relationship of this episode to the original exposure remains unclear. At this time, social and occupational functioning remains within normal limits. There is no evidence of neurological or cognitive dysfunction or other physical decline. The patient remains actively employed and successful as a research scientist.

Discussion

The case presented here offers compelling evidence of transient neuropsychological deficits following prolonged, intense occupational laboratory exposure to *P. piscicida* cultures. In this specific case, there exists a relative certitude of the relationship of exposure to *Pfiesteria* cultures to the initially documented health effects. Further, the constellation of findings was consistent with that often observed as a result of neurotoxin exposures, with the implication of direct or indirect frontal–temporal system involvement. The marked neurocognitive deficits largely resolved within 6 weeks of exposure cessation, with only minimal residual. Although the development of neurological symptoms 9 years after exposure cessation is intriguing, it is of course less compelling with regard to causation, given the lack of temporal relatedness to exposure. Nonetheless, these symptoms raise the question of long-term sequelae or vulnerability to other illnesses, and these potential long-term exposure effects of *Pfiesteria* are an area also presently under investigation.

Numerous other occupational laboratory and civilian cases have presented to the MDC of the Bryan ADRC for examination since this initial case. Table 1 is a summary of some of the findings of this small clinical series. The clinical variation across cases is noteworthy, particularly in the laboratory exposure cases where there was known and proven exposure to *Pfiesteria* cultures. There were often secondary or preexisting medical conditions that are commonly encountered in the general population. These included reactive airway disease and sinusitis, obstructive sleep apnea, and chronic fatigue. Such complicating medical conditions were also present in the civilian cases. The possibility of unconscious modeling existed in nearly all cases

after the first two affected laboratory exposures, given the high publicity of *Pfiesteria* in the lay and scientific press. Finally, possible malingering or secondary gain (e.g., attention, secondary gain) certainly could be a factor and was a leading suspicion in the symptomatology of one occupational civilian case despite a convincing account of initial exposures. The presence of confounding medical illness, the exclusion or accounting for other toxic exposures (e.g., alcohol or drug), the presence of confounding psychiatric factors or illness, and clinical variability are all commonly encountered in the evaluation of toxic exposure cases and do not preclude an added effect due to the putative environmental agent. This situation suggests that well-designed prospective epidemiological investigation will be necessary to clearly delineate *Pfiesteria* neurotoxic effects. Other marine-associated illness and other adverse health properties of estuarine waters must be considered and carefully accounted for.

Further contributions to this variation likely include possible differences in toxin(s) (e.g., *Pfiesteria* subspecies and life cycles) as well as differences in exposure route, intensity, and duration. Molecular targets and target organs and tissues have yet to be delineated as well as the process of toxin(s) metabolism, storage, and excretion. Downstream effects of toxin(s) on tissue integrity, nuclear or mitochondrial integrity, or endogenous protein targets are also possible. The possible priming mechanism of repeated or long-term exposures is unclear. The distinct possibility of sensitization to antigens associated with *Pfiesteria* and/or its toxins should be considered given the repeated exposures, and neuroimmune mechanisms might account for some or all of the acute or chronic health effects. In no known human laboratory case has it been shown that a first-time, single-pulse exposure to *Pfiesteria* organism or toxin(s) has resulted in health effects. The biological exceptions to this would presumably be the acute stunning and killing of fish by *Pfiesteria* exposure and reports of civilian case illness. However, the mechanism of fish killing and the putative

toxin(s) involved may or may not be related to mechanisms of human health effects.

The current cases do not clarify whether the CNS effects are primary or secondary results of the clear-cut systemic exposure (inhalational and dermal) and systemic signs (skin lesions, possible liver or renal dysfunction). Of course, host factors must also be considered, as each individual also embodies potentially predisposing genetic, medical (e.g., host immune mechanisms), psychiatric, and environmental factors that also likely influence outcome.

Naturally, efficient and systematic investigation of these factors relies on identification of a biomarker. Until such time, full elucidation of *Pfiesteria* health effects will be greatly hindered. Although public education is critical, scientific progress would be greatly assisted if dissemination of information about the potential human health effects of exposure could proceed in an orderly and organized manner that discourages symptom exaggeration and reassured the general public through accurate accounts of *Pfiesteria* research findings. Personal and external sources of bias must be eliminated to the extent feasible.

Despite the considerable present ambiguity surrounding *Pfiesteria* and its exposure sequelae, the evidence of potential health effects suggested by complaints (7), objective clinical cases, and various published case series indicate that use of precautionary measures and thorough examination of exposed persons is warranted. Evaluation of persons with alleged exposures to *P. piscicida* should ideally involve a thorough, rapid assessment. As Grattan has noted, all suspect cases should be considered and evaluated seriously and thoroughly (8). Because of the variation in symptom development and manifestations, a multidisciplinary approach involving internal medicine, neurology, neuropsychology, and occupational health will likely yield the most productive data. Toward this end, storage of acute and convalescent plasma and serum samples, skin biopsies, fat biopsies, and bile samples would also be ideal in anticipation of

Table 1. Signs and symptoms of laboratory- and environment-exposed cases.

	Laboratory cases					Environmental cases			
	1	2	3	4	5	1	2	3	4
Physical complaints	Y	Y	Y	Y	Y	Y	Y	Y	Y
Memory complaints	N	Y	Y	Y	Y	Y	Y	Y	N
Mood/personality changes	N	Y	Y	Y	Y	N	Y	N	Y
Abnormal neurological	Y	Y	N	N	Y	–	Y	Y	N
Abnormal physical exam (skin)	N	Y	N	N	N	Y	N	N	Y
Abnormal neuroimaging/labs	N	Y	N	N	Y	–	N	Y	N
Confounding medical factors	N	N	Y	N	Y	–	Y	Y	Y
Confounding psychiatric factors	N	N	Y	N	Y	Y	Y	N	Y
Possible exposure effects	Y	Y	?	?	?	–	–	–	–
Potential modeling	N	N	Y	Y	Y	N	Y	N	Y
Possible EAS	–	–	–	–	–	Y	?	?	?

Abbreviations: N, no; Y, yes; –, not applicable; ?, equivocal.

the development of biomarkers or specific assays for toxin(s). These could be used for surrogate markers (e.g., inflammatory cytokines, chemokines) that might index exposure. The differential diagnosis is clearly affected by the age and health status of the person. Exclusion of competing etiologies such as psychiatric illness, alcohol or drug abuse, dementia or neurodegenerative illness, small vessel cerebrovascular disease, and other toxic causes is critical to narrow the spectrum of symptoms associated with exposure. In addition, study of those many persons with known or suspected concomitant illnesses is also essential, as they may be at greater risk for developing illness as a result of exposure.

Management should promote exposure cessation and close medical follow-up by primary care and relevant specialists. Findings from the case presented here and the series followed in Maryland (*3*) suggest resolution of symptoms in cases of acute exposure. However, the symptom duration and long-term effects in cases of low-level chronic exposure remain unknown. Variations in exposure route, intensity, and duration as well as the previously noted factors possibly contributing to presentation variability likely affect the rate of resolution of illness as well.

The Centers for Disease Control has put forth criteria for possible estuary associated syndrome (PEAS) (*9*). At present, a diagnosis of PEAS requires that a healthcare provider has ruled out other competing causes for symptoms. We propose an expansion of this classification system of estuary associated syndrome (EAS) to allow for varying degrees of certainty. Specifically, following numerous examples of other illness and disease states (e.g., Alzheimer's disease) (*10*), discriminating between possible, probable, and definite EAS

would enhance the sophistication of the clinical designation and allow for the presence of other contributing factors. Probable EAS would require the absence of competing factors, proved exposure to *Pfiesteria* organisms or associated fish kills, and adherence to a defined set of clinical symptoms including neurocognitive symptoms and signs. Of course, a diagnosis of definite EAS awaits identification of a reliable, validated biomarker. However, differentiation between possible and probable EAS could be made on the bases of symptom constellations, temporal relationship to exposure, and presence or absence of competing diagnoses. Further, identification of laboratory versus environmental cases may also aid rapid and clear communication of information, particularly given that occupational laboratory exposure cases may differ radically from putative ongoing occupational civilian (e.g., workers in estuarine environments) or civilian exposures.

In summary, we present a case involving the most prolonged and intense human exposure to *P. piscicda* cultures known, with significant associated neurological illness and subsequent symptom resolution. Recurrence of significant neurological illness 6 years after the exposure may represent a directly associated long-term effect despite cessation of exposures, an increased vulnerability to other diseases of the nervous system, or be totally unrelated. As this second illness was not proved to be multiple sclerosis (the most likely diagnosis given the presentation) and no other cause was identified, it was considered more likely than not to be related to the initial occupational laboratory exposure. On a scientific basis, we cautiously assert a probable causative link between this known massive exposure and the associated primary initial

illness and a possible causative or contributory link to the secondary delayed illness, and look forward to the biological and physiological scientific progress that will facilitate rigorous, systematic research of human health sequelae. In the interim, we are convinced of the need to be diplomatically responsive to public health concern, to collect data to the extent feasible, and to provide maximal clinical care to those seeking medical assistance.

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