The Prooxidant State and Psychologic Stress

We have read with interest the work by Lesgards et al. (*1*) on the effect of different lifestyle factors on their test system, which measures the resistance of red blood cells to an oxidative challenge. Their study showed that psychologic stress is a major factor influencing antioxidant status. This finding encouraged us to share our experiences of a earlier study that resulted in similar conclusions using an enzyme, 5′-ectonucleotidase (NT), located on the external surface of lymphocytes (*2,3*). NT is significant because it has a role in lymphocyte maturation; newborns with persistently low activities have multiple infections, whereas with normalization of NT, their infections resolved (*4*). We previously showed that NT activities decreased, by then-unknown mechanism(s), as the course of HIV (human immunodeficiency virus) infection progressed (*5*). Later, we carried out this study in psychologically stressed patients to see if stress itself could lower the activity of this enzyme and thereby contribute to the immune deficiency reported for stressed/depressed patients (*6*). In one aspect of this study, honors students in psychology were monitored at different times of the year when their stress levels were low (after holidays) and high (exams/thesis writing). These students were psychologically assessed for stress using the Profile of Mood Score (POMS). The POMS scores were significantly correlated with NT, which was significantly lower (30%) at high stress periods and normalized when the stress resolved (after holidays), indicating the reversibility of the effect of stress on NT.

In another group of chronically stressed/depressed patients, NT values were also found to be very low; however, in a subgroup of these patients taking vitamins A, C, and E and coenzyme Q10, the NT values were equivalent to those of an unstressed healthy population (*2*). Subsequent *in vitro* studies showed that NT was inhibited within 5 min after exposure to superoxide anions and that this effect could be reversed either by ascorbate added to the *in vitro* system at physiologic levels present in serum (100 µM) or by oral administration of the same antioxidant mix to volunteers for about 6 weeks (*3,7*). Measurement of tissue ascorbate also showed that this metabolite, like NT, decreased significantly at high-stress periods and resolved when the stress dissipated. Our earlier findings of low NT in HIV-positive patients further confirmed our oxidant mechanism when these patients were later shown to have a high prooxidant state (*8*).

Besides stress, we also studied another prooxidant state—diabetes—and showed that with poor management of this condition, as measured by hemoglobin A1C (HbA1C), NT decreased significantly (*7*). That is, NT correlated negatively and significantly with HbA1C. These studies, taken together, confirmed that NT is a good indicator of a high prooxidant state, whether through HIV infection, psychologic stress, or diabetes.

In summary, psychologic stress gives rise to a prooxidant state that is reflected by low NT and increased red blood cell sensitivity to oxidative insult. Why stress should cause a prooxidant state is not known. We hypothesize that stress by increasing cortisol favors the development of an innate immune response (*2,3*). In normal circumstances the innate response would abate as the adaptive immune system takes over. However, with chronic stress the high prooxidant nature of the innate response persists, because of chronically elevated cortisol, and NT and probably other important extracellular proteins of lymphocytes are inhibited, resulting in an attenuated adaptive immune response and subsequent immunodeficiency. That an innate or proinflammatory process persists in stressed patients is evidenced by persistent increased blood concentrations of acute phase proteins seen in these patients (*9,10*).

Ainsley H. Chalmers
Flinders University of South Australia
Bedford Park, South Australia
E-mail: chalmers.ah@flinders.edu.au
Jane S. Blake-Mortimer
University of Adelaide
Adelaide, South Australia
Anthony H. Winefield
University of South Australia
Adelaide, South Australia

References and Notes

1. Lesgards J-F, Durand P, Lassarre M, Stocker P, Lesgards G, Lanteaume A, Prost M, Lehucher-Michel M-P. Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects. Environ Health Perspect 110:479–486 (2002).
2. Blake-Mortimer JS, Winefield AH, Chalmers AH. The relationship between 5′-ectonucleotidase and psychological stress. Int J Stress Manage 3:189–207 (1996).
3. Blake-Mortimer JS, Winefield AH, Chalmers AH. Evidence for free radical-mediated reduction of lymphocytic 5′-ectonucleotidase during stress. Int J Stress Manage 5:57–75 (1998).
4. Bastian JF, Ruedi JM, MacPherson GA, Golembsky HE, O'Connor RD, Thompson LF. Lymphocyte ecto-5′-nucleotidase activity in infancy: increasing activity in peripheral blood B cells precedes their ability to synthesize IgG in vitro. J Immunol 132:1767–1772 (1984).
5. Chalmers AH, Hare C, Wooley G, Frazier IH. Lymphocyte ectoenzyme activity compared in healthy persons and patients seropositive to or at high risk of HIV infection. Immunol Cell Biol 68:81–85 (1990).
6. Cohen S, Miller GE, Rabin BS. Psychological stress and antibody response to immunization: a critical review of the human literature. Psychosom Med 63:7–18 (2001).
7. Chalmers AH, Blake-Mortimer JS, Winefield AH. Lymphocytic 5′-ectonucleotidase, an indicator of oxidative stress in humans. Redox Report 5:89–91 (2000).
8. Allard JP, Aghdassi E, Chau J, Salit I, Walmsley S. Oxidative stress and plasma antioxidant micronutrients in humans with HIV infection. Am J Clin Nutr. 67:143–147 (1998).
9. Maes M. A review on the acute phase response in major depression. Rev Neurosci 4:407–416 (1993).
10. Joyce PR, Hawes CR, Mulder RT, Sellman JD, Wilson DA, Boswell DR. Elevated levels of acute phase plasma proteins in major depression. Biol Psychiatry 32:1035–1041 (1992).

The Prooxidant State and Psychologic Stress: Response to Chalmers et al.

We thank Chalmers et al. for their interest in our paper published in *EHP* (*1*). We also thank them for sharing their earlier studies on free radical-mediated reduction of lymphocytic 5′-ectonucleotidase (NT) activity during psychologic stress (*2–4*) and on the reduction of this activity during HIV infection and diabetes (*4,5*), which were reported to be associated with a high prooxidant state.

Their works have actually shown that psychologic stress results in a decreased NT activity and in a decreased tissue ascorbate that can both be normalized by antioxidant intake or when the stress resolved. Thereby, Chalmers et al. suggest that psychologic stress itself can cause an increased prooxidant state responsible for decreased NT activity, which may contribute to the immune deficiency reported in stressed or depressed patients. They thus propose that a decrease in NT activity may be a good marker of a prooxidant state in humans (*2–4*).

In agreement with their hypothesis, our population-based study underlined that, independently of other studied factors and in particular of tobacco smoking, psychologic stress truly contributes to a decrease in the overall antioxidant capacity of healthy subjects as measured by means of the free radical-mediated hemolysis test (Spiral's KRL test) (*6,7*). Furthermore, our results indicated that among the studied factors and besides cigarette smoking, psychologic stress was the lifestyle factor that was most markedly associated with a decreased antioxidant capacity (*1*). Thus, in accordance with reports from Chalmers et al. (*5*), Niki (*8*) and Young and Woodside (*9*) suggest that psychologic stress can induce per se an unbalanced antioxidative homeostasis leading to the occurrence of oxidant stress that may increase the incidence of free radical-mediated diseases such as cancer, cardiovascular diseases, diabetes, inflammatory diseases, and HIV infection.

It is noteworthy that the aim of our study was to evaluate lifestyle factors that may contribute to the normal variability of the overall antioxidant status in healthy subjects. Because of the involvement of numerous studied lifestyle factors in the variability of the overall antioxidant status evaluated in our study, we did not thoroughly discuss oxidant stress probably caused by psychologic stress or emphasize the major importance of the potential involvement of psychologic stress in human disease. Although we have not previously mentioned

the works of Chalmers et al. on the psychologic stress-mediated prooxidant state, we hope that these letters will highlight their studies.

Concerning the mechanism underlying psychologic stress-related oxidant stress described by Chalmers et al., we agree with their hypothesis of the involvement of a chronic elevation of cortisol favoring the development of an innate immune response at the expense of the humoral immune system, which results in chronic inflammatory process and therefore in a high prooxidant state. Whether the inhibition of cortisol action as compared to antioxidant therapy could reduce the prooxidant state induced by psychologic stress remains to be determined. Moreover, according to Chalmers et al. (*4*), lymphocytic NT activity appears to be a good marker of oxidant stress. Additional clinical designs are now needed to confirm the use of this assay as a biomarker of a prooxidant state. The use of the hemolysis test in combination with analytic dosages of specific biomarkers of antioxidant and prooxidant states could be helpful for this purpose.

In conclusion, in light of Chalmers and coworkers' findings (*3,4*) and of our own findings (*1*), psychologic stress effects, which are often underestimated, should be taken into account among healthy control subjects and among patients bearing free radical-mediated disease in clinical and epidemiologic trials. In particular, regarding Chalmers et al.'s study that showed a reduced NT activity probably related to oxidant stress during HIV infection (*5*), it should be determined whether psychologic stress is responsible for an oxidant stress-mediated increase in immune deficiency resulting from HIV infection. Indeed, investigations on the potential involvement of psychologic stress in human free radical-mediated and degenerative disease are still too scarce, and Chalmers et al. are right in emphasizing the major potential influence of psychologic stress.

Phillipe Durand
Centre Européen de Recherche
et d'Analyse
Dijon, France
Marie-Pascale Lehucher-Michel
Consultation de Hospitalière de Pathologies
Professionnelles
Service de Médecine du Travail
Marseille, France
E-mail: mlehucher@ap-hm.fr

References and Notes

1. Lesgards J-F, Durand P, Lassarre M, Stocker P, Lesgards G, Lanteaume A, Prost M, Lehucher-Michel M-P. Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects. Environ Health Perspect 110:479–486 (2002).
2. Blake-Mortimer JS, Winefield AH, Chalmers AH. The relationship between 5′-ectonucleotidase and psychological stress. Int J Stress Manage 3:189–207 (1996).
3. Blake-Mortimer JS, Winefield AH, Chalmers AH. Evidence for free radical-mediated reduction of lymphocytic 5′-ectonucleotidase during stress. Int J Stress Manage 5:57–75 (1998).
4. Chalmers AH, Blake-Mortimer JS, Winefield AH. Lymphocytic 5′-ectonucleotidase, an indicator of oxidative stress in humans. Redox Report 5:89–91 (2000).
5. Chalmers AH, Hare C, Wooley G, Frazier IH. Lymphocyte ectoenzyme activity compared in healthy persons and patients seropositive to or at high risk of HIV infection. Immunol Cell Biol 68:81–85 (1990).
6. Prost M. Process for the determination by means of free radicals of the antioxidant properties of a living organism or potentially aggressive agents. U.S. patent no. 5,135,850. Washington, DC:U.S. Patent and Trademark Office, 1990. Available: http://patft.uspto.gov/netacgi/nph-Parser?patentnumber=5135850 [cited 18 November 2002].
7. Blache D, Prost M. Free radical attack: biological test for human resistance capability. In: Proceedings of the IX College Park Colloquium on Chemical Evolution: A Lunar-Based Chemical Analysis Laboratory (Ponnamperuma C, Gehrke W, eds). Washington, DC:NASA, 1992;82–98.
8. Niki E. Free radicals in the 1900's: from in vitro to in vivo. Free Radic Res 33:693–704 (2001).
9. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 54:76–86 (2001).

Neuropsychologic Testing versus Visual Contrast Sensitivity in Diagnosing PEAS

I would like to comment on Hudnell and Shoemaker's response (Hudnell and Shoemaker 2002) to our letter (Swinker and Burke 2002), both published in the March 2002 issue of *EHP*.

In their letter Hudnell and Shoemaker (2002) asserted that "neuropsychologic test deficits are nonspecific." In the hands of trained neuropsychologists, specific areas of functional deficits can be identified. Attention, executive function, short-term memory, mood, adjustment, and verbal or visual fluency can be specifically assessed. Convergent validity can be established by evaluating functioning in a number of ways, that is, different test instruments that measure the same thing. Neuropsychologic deficits of a specific nature with characteristic patterns have been found in response to certain environmental exposures. The use of neuropsychologic testing has been validated in a variety of clinical settings and is not in dispute.

Memory problems have been the most unique and significant symptoms reported following *Pfiesteria* exposure in both the laboratory setting (Glasgow et al. 1995) and in the 1997 Maryland environmental cohort (Grattan et al. 1998). This memory deficit was objectified and quantified in Maryland by use of neuropsychologic testing. Significant abnormalities were observed on three specific tests; the Rey Auditory Verbal Learning Test was the most consistently and significantly affected. Other tests batteries, which measured other neuropsychologic functions, were not affected in the same way. Therefore, the Maryland neuropsychologic findings were specific. The deficits were not global. Their subjects' complaints did correlate with the specific test performance observed.

Use of visual contrast sensitivity (VCS) testing in lieu of neuropsychologic testing in the context of possible estuary associated syndrome (PEAS) is very much in dispute. Shoemaker and Hudnell (2001) offer no evidence to support their assertion that VCS can be used in place of neuropsychologic testing in the evaluation of PEAS. Until the VCS test is validated in direct comparison to the neuropsychologic testing that has been demonstrated to be sensitive to PEAS, advocacy of the test remains in the realm of speculation.

Other authors have echoed our reservations about the use of VCS over the past year. According to Rubin et al. (2001),

> VCS testing is controversial because abnormal findings are not toxin-specific and abnormal findings can be interpreted only cautiously unless accompanied by other fairly extensive visual tests.

Morris (2001) also commented that

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

Whether all the hotline callers in our 1997 study (Swinker et al. 2001) are considered exposed or unexposed depends on whether the estuary associated syndrome criteria for 1997 [Centers for Disease Control and Prevention (CDC) 1997] or the more liberal criteria from 1999 (CDC 1999) are used. Since PEAS is the current appellation and the one that Hudnell and Shoemaker discuss (Shoemaker and Hudnell 2001), then all the hotline callers should remain grouped together because they are all "exposed" under the PEAS criteria. The current PEAS case description includes the stipulation that "a health care provider cannot identify another cause for the symptoms" (CDC 1999). This type of analysis was included in our work as an accepted—and expected—research practice. [A similar process of analysis was also done in the original Maryland work, where cases were defined based on documented exposure to estuary waters and abnormal performance on neuropsychologic testing that could not be explained otherwise (Grattan et al. 1998).] Our hotline callers with neuropsychologic test

impairment had a variety of well-accepted medical explanations for their symptoms or test performances; there was no need or justification to postulate a new syndrome (Swinker et al. 2001). They had no unique or consistent pattern of abnormality on the Rey Auditory Verbal Learning Test or any other test battery. Their performance was affected across a variety of functional areas, not in a single area. The neuropsychologic test performances of the callers as a group and of their asymptomatic controls were similar (Swinker et al. 2001). Thus we have not "dismissed the diagnoses of estuary-associated syndrome" (Hudnell and Shoemaker 2002). We have subjected the data to scientific analysis before forming our conclusions.

Hudnell's VCS data (Hudnell and Shoemaker 2002) suggest that the performance of the hotline control group (10 persons without symptoms) in our original work (Swinker et al. 2001) was also abnormal. This finding suggests that VCS deficits may be highly prevalent and so nonspecific as to be of limited value as a diagnostic test. VCS is affected by many factors: chemical exposures (e.g., mercury or solvents), congenital conditions (e.g., dyslexia or other learning disabilities), degenerative conditions (e.g., Parkinson's disease and multiple sclerosis), ocular disease (e.g., glaucoma or macular degeneration), other conditions [e.g., AIDS or cystic fibrosis (Hudnell 1998)], and the use of alcohol or medications (e.g., antiepilepsy drugs).

Assessment of hydrogen sulfide exposure in estuary fishermen was included in a multiyear longitudinal cohort study recently conducted in North Carolina (Moe et al. 2001). VCS and neuropsychologic testing were also systematically performed at regular intervals as part of this investigation. The data analysis phase of this effort has just begun. We should soon know more about the frequency of hydrogen sulfide exposure in estuary watermen as well as any relationship or correlation between VCS and neuropsychologic test performance in watermen over the seasons and the years.

Finally, subsequent to the CDC report (CDC 2000) quoted in my letter (Swinker and Burke 2002), the state of Maryland has verified five PEAS cases in their ongoing surveillance, as noted by Hudnell and Shoemaker (2002). These cases have been reported to the CDC (Backer et al. 2001) with little fanfare and detailing some methodologic concerns regarding exposure assessment. I do not know whether any of Hudnell and Shoemaker's cases are included in those five cases.

Marian Swinker
East Carolina University School of Medicine
Greenville, North Carolina
E-mail: swinkerm@mail.ecu.edu

References

Backer LC, Niskar AS, Rubin C, Blindauer K, Christianson D, Naeher L, Rogers HS. 2001. Environmental public health surveillance: possible estuary-associated syndrome. Environ Health Perspect 109(suppl 5):797–801.

Centers for Disease Control and Prevention (CDC). 1997. Results of the Public Health Response to *Pfiesteria* Workshop—Atlanta, Georgia, September 29–30, 1997. MMWR Morb Mortal Wkly Rep 46:951–952.

———.1999. Possible estuary-associated syndrome. MMWR Morb Mortal Wkly Rep 48:381–382.

———. 2000. Surveillance for possible estuary-associated syndrome—six states, 1998-1999.MMWR Morb Mortal Wkly Rep 49:372–374.Glasgow H, Schmechel D, Tester P, Rublee P. 1995. Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. J Toxicol Environ Health 46:501–522.

Grattan L, Oldach D, Perl TM, Lowitt MH, Matuszak DL, Dicson C, et al. 1998. Learning and memory difficulties after environmental exposure to waterways containing toxin-producing *Pfiesteria* or *Pfiesteria*-like dinoflagellates. Lancet 352:532–539.

Hudnell HK, Shoemaker RC. 2002. Visual contrast sensitivity: response [Letter]. Environ Health Perspect 110:A121–A123.

Hudnell K. 1998. Human Visual Function in the North Carolina Clinical Study on *Pfiesteria piscicida*. EPA 600-R98-132. Research Triangle Park, NC:U.S. Environmental Protection Agency.

Moe C, Turf E, Oldach D, Bell P, Hutton S, Savitz D, et al. 2001. Cohort studies of health effects among people exposed to estuarine waters: North Carolina, Virginia, and Maryland. Environ Health Perspect 109(suppl 5):781–786.

Morris JG. 2001. Human health effects and *Pfiesteria* exposure: a synthesis of available clinical data. Environ Health Perspect 109(suppl 5):787–790.

Rubin C, McGeehin M, Holmes A, Backer L, Burrison G, Earley M, et al. 2001. Emerging areas of research reported during the CDC National Conference on *Pfiesteria*: From Biology to Public Health. Environ Health Perspect 109(suppl 5):633–637.

Shoemaker RC, Hudnell HK. 2001. Possible estuary-associated syndrome: symptoms, vision, and treatment. Environ Health Perspect 109:539–545.

Swinker M, Burke W. 2002. Visual contrast sensitivity as a diagnostic tool [Letter]. Environ Health Perspect 110:A120–A121.

Swinker M, Koltai D, Wilkins J, Stopford W. 2001. Is there an estuary-associated syndrome in North Carolina? Findings in a series of hotline callers. NC Med J 12:126–132.

Neuropsychologic Testing versus Visual Contrast Sensitivity: Response

We appreciate the opportunity to respond to Swinker's letter regarding our response (Hudnell and Shoemaker 2002) to her previous letter (Swinker and Burke 2002). Her previous letter concerned our first *EHP* article (Shoemaker and Hudnell 2001) on possible estuary-associated syndrome (PEAS). Her current letter raises five issues: *a*) "nonspecificity" of visual contrast sensitivity (VCS) and neuropsychologic tests; *b*) memory loss being the most unique and significant effect in PEAS; *c*) validity of VCS as an indicator of neurologic deficits in PEAS cases; *d*) VCS deficits in Swinker's 10 hotline caller controls; and *e*) hydrogen sulfide exposure in North Carolina estuaries.

Concerning "nonspecific" tests, in their previous letter Swinker and Burke (2002) objected to our use of VCS tests in PEAS investigations because VCS deficits are "nonspecific." As noted in our *EHP* articles on PEAS (Shoemaker 2001; Shoemaker and Hudnell 2001) and in our response to her first letter (Hudnell and Shoemaker 2002), VCS deficits are "nonspecific" because they have multiple possible etiologies. In her current letter Swinker objects to our response that "neuropsychologic test deficits are nonspecific," reasoning that neuropsychologic tests identify "specific areas of functional deficits." We maintain that tests which detect functional deficits associated with impairment in specific neurologic pathways are still "nonspecific" tests if there is more than one possible etiologic agent. Our position is consistent with the definition of "nonspecific" in *Dorland's Illustrated Medical Dictionary* (Dorland 1988): "1. not due to any single known cause, as to a particular pathogen." We stand by our statement and maintain that both VCS and neuropsychologic tests *a*) are "nonspecific" because deficits have multiple possible causes; *b*) can reveal impairment in specific neurologic pathways; *c*) are useful objective indicators of neurologic impairment in a syndrome otherwise described only by "nonspecific" symptoms and; *d*) require investigation of alternative explanations of deficits because the tests are "nonspecific."

Swinker stated that

> Memory problems have been the most unique and significant symptoms reported following *Pfiesteria* exposure in both the laboratory setting (Glasgow et al. 1995) and in the 1997 Maryland environmental cohort (Grattan et al. 1998)

This statement is not supported by those reports or the experience of clinicians who have recognized PEAS cases. Memory problems are not unique to PEAS, and cases reported many symptoms, each of which can cause a significant decline in quality of life. The laboratory worker most severely affected by *Pfiesteria* exposure reported 13 multiple-system symptoms (Glasgow et al. 1995). He recovered memory function over many months, but developed unilateral blindness for which *Pfiesteria* exposure was considered the most likely cause (Schmechel and Koltai 2001). The Maryland study reported higher percentages of 10 symptoms in exposed participants than in control participants (Grattan et al. 1998). Memory loss was not a unique symptom category in that study, but was lumped with confusion, disorientation, and concentration difficulty (Grattan et al. 1998). Shoemaker's 37 PEAS cases reported 18 diverse symptoms, 13 of which were reported by more than one-third of the cases, and VCS was reduced by 60% in cases relative to controls (Shoemaker 2001). In her current letter, Swinker further states that the Maryland neuropsychologic results were specific for memory loss and that "deficits were not global." The Maryland study actually reported statistically significant neuropsychologic deficits in fine motor speed, dexterity, resistence to interference, and selective attention in addition to memory loss (Grattan

et al. 1998). This evidence suggests that PEAS is a distinct syndrome characterized by multiple-system symptoms and a variety of neurologic effects, including vision and memory loss.

Swinker states that VCS has not been validated for use in helping to diagnose PEAS. We submit that many steps have been taken toward validation. Impetus for investigating the validity of VCS in PEAS diagnosis came from earlier observations of VCS deficits in populations contacting *Pfiesteria*-inhabited estuaries, relative to populations without exposure potential (Hudnell 1998; Hudnell et al. 2001; Turf et al. 1999). The group differences in VCS were independent of age, smoking, and bright sunlight exposure, and the groups did not appear to differ in exposures to solvents, pesticides, metals, or fumes. The absence of group differences in visual acuity suggested that neurologic, rather than optical, factors likely caused the VCS deficits. The data further indicated that the VCS deficits were related to total time spent at fish kills in the past. These results suggested that the VCS deficit might be a persistent sign of a neurologic effect from *Pfiesteria* toxin(s) or other factors associated with estuarine contact (Hudnell 1998; Hudnell et al. 2001; Swinker et al. 2001a). Our subsequent *EHP* articles reported the initial data on validity of VCS as an indicator of neurologic impairment in cases meeting the Centers for Disease Control and Prevention (CDC) criteria for PEAS (Shoemaker 2001; Shoemaker and Hudnell 2001). The Grand Rounds cases (Shoemaker and Hudnell 2001) showed large VCS deficits during illness, and VCS recovery coincident with symptom resolution after cholestyramine treatment during acute, chronic, and repeated-acquisition of PEAS. These associations between VCS, symptoms, and treatment were verified in Shoemaker's subsequent cohort study and double-blinded, placebo-controlled crossover trial (Shoemaker 2001). Control conditions ruled out several possible causes of VCS fluctuations; VCS remained stable over time in healthy individuals, VCS was not altered by cholestyramine therapy in hypercholesterolemia patients who did not have PEAS, and PEAS cases showed VCS recovery and symptom resolution with cholestyramine, but not placebo, therapy (Shoemaker 2001). The VCS deficits in PEAS cases did not appear to be caused by solvent exposures. The group average VCS spatial-frequency profile in the PEAS cases (Shoemaker 2001) showed greatest reduction at midspatial frequency, similar to that seen following chronic solvent exposure (Hudnell 1998). Solvent exposure cases, however, did not respond to cholestyramine therapy (Shoemaker RC. Unpublished data), and review of the literature revealed no reports of therapy that were effective at resolving solvent-induced VCS deficits. Ocular abnormalities and diseases involving neurologic function also were probably not the cause of VCS deficits in the PEAS cases. None of Shoemaker's active PEAS cases received treatment for these conditions during the time of cholestyramine therapy and VCS recovery (Shoemaker 2001; Shoemaker and Hudnell 2001). Each of these observations is an important step in the process of validating VCS for use in PEAS diagnosis. Further validation may come from the demonstration of correlations between VCS and neuropsychologic data during illness and recovery with treatment in 17 of Shoemaker's PEAS cases who submitted to neuropsychologic testing (Shoemaker RC. Unpublished data).

In her letter, Swinker claims that VCS deficits in the hotline controls suggest "that VCS deficits may be highly prevalent and so nonspecific as to be of limited value as a diagnostic test." Her 10 local government-employee controls all reported exposure to *Pfiesteria*-inhabited estuaries and/or organic solvents, risk factors for VCS deficits (Hudnell and Shoemaker 2002). Although Swinker claims that the controls were "asymptomatic" in her current letter, neither her report (Swinker et al. 2001b) nor our review of the original data (Hudnell HK. Unpublished data) indicated that symptoms were assessed in the control group. Swinker's speculation on the prevalence of VCS deficits based on a small sample with confounding factors and incomplete assessment is unfounded. The consistent ability of studies to show VCS differences between exposed and control cohorts suggests that VCS deficits are not common in control populations screened for risk factors such as neurotoxic exposure and neurologic disease (Hudnell et al. 1996).

Finally, Swinker proposed that hydrogen sulfide exposure in estuarine waters may cause VCS deficits (Swinker and Burke 2002) and implied that this hypothesis would be assessed in the North Carolina longitudinal cohort study. The North Carolina study did not include measurements of hydrogen sulfide or any other potentially neurotoxic compound (Moe et al. 2001). Questions on odor detection were included but are not sufficient to address the hypothesis. Hydrogen sulfide exposures at concentrations and durations that may impact VCS are unlikely to be encountered in the open estuaries frequented by watermen.

We look forward to additional clarification of these issues in peer-reviewed research articles.

This letter was reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation.

H. Kenneth Hudnell
U.S. Environmental Protection Agency
ORD/NHEERL/NTD/NPBT
Research Triangle Park, North Carolina
E-mail: hudnell.ken@epamail.epa.gov
Ritchie C. Shoemaker
Center for Research on Biotoxin-Associated Illness, Inc.
Pocomoke City, Maryland

References

Dorland WA. 1988. Dorland's Illustrated Medical Dictionary. 27th ed. Philadelphia:W.B. Saunders, 1148.

Glasgow HB, Burkholder JM, Schmechel DE, Tester PA, Rublee PA. 1995. Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. J Toxicol Environ Health 46:501–522.

Grattan LM, Oldach D, Perl TM, Lowitt MH, Matuszak DL, Dickson C, et al. 1998. Learning and memory difficulties after environmental exposure to waterways containing toxin-producing *Pfiesteria* or *Pfiesteria*-like dinoflagellates. Lancet 352:532–539.

Hudnell HK, Otto DA, House DE. 1996. The influence of vision on computerized neurobehavioral test scores: a proposal for improving test protocols. Neurotoxicol Teratol 18:391–400.

Hudnell HK. 1998. Human visual function in the North Carolina study on *Pfiesteria piscicida*. U.S. EPA # 600-R-98-132. For the North Carolina Department of Health and Human Services and the North Carolina Task Force on *Pfiesteria*. Chapel Hill, NC:University of North Carolina School of Public Health.

Hudnell HK, House D, Schmid J, Koltai D, Wilkins J, Stopford W, et al. 2001. Human visual function in the North Carolina clinical study on possible estuary associated syndrome. J Toxicol Environ Health A 62:575–594.

Hudnell HK, Shoemaker RC. 2002. Visual contrast sensitivity: response [Letter]. Environ Health Perspect 110:A121–A123.

Moe CL, Turf E, Oldach D, Bell P, Hutton S, Savitz D, et al. 2001. Cohort studies of health effects among people exposed to estuarine waters: North Carolina, Virginia, and Maryland. Environ Health Perspect 109(suppl 5):781–786.

Schmechel DE, Koltai DC. 2001. Potential human health effects associated with laboratory exposures to *Pfiesteria piscicida*. Environ Health Perspect 109(suppl 5):775–779.

Shoemaker RC. 2001. Residential and recreational acquisition of possible estuary-associated syndrome: a new approach to successful diagnosis and treatment. Environ Health Perspect 109(suppl 5):791–796.

Shoemaker RC, Hudnell HK. 2001. Possible estuary-associated syndrome: symptoms, vision and treatment. Environ Health Perspect 109:539–545.

Swinker M, Burke WA. 2002. Visual contrast sensitivity as a diagnostic tool [Letter]. Environ Health Perspect 110:A120–A121.

Swinker M, Koltai D, Wilkins J, Hudnell K, Hall C, Darcey D, et al. 2001a. Estuary-associated syndrome in North Carolina: an occupational prevalence study. Environ Health Perspect 109:21–26.

Swinker M, Koltai D, Wilkins J, Stopford W. 2001b. Is there an estuary associated syndrome in North Carolina? Findings in a series of hotline callers. NC Med J 62:126–132.

Turf E, Ingsrisawang L, Turf M, Ball JD, Stutts M, Taylor J, et al. 1999. A cohort study to determine the epidemiology of estuary-associated syndrome. Virginia J Sci 50:299–310.