August 12, 1998 FINAL DRAFT

# HUMAN VISUAL FUNCTION IN THE NORTH CAROLINA CLINICAL STUDY ON *PFIESTERIA PISCICIDA*

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**Manuscript prepared at the request of the the North Carolina Department of Health and Human Services for review by the North Carolina Task Force on *Pfiesteria* (William Roper, Chairman)**

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## ABSTRACT

**Human Visual Function in the North Carolina Clinical Study on *Pfiesteria piscicida*.** Hudnell, H.K., U.S. EPA, RTP, NC. The U.S. Environmental Protection Agency assisted the North Carolina Department of Health and Human Services in conducting a human-health study to investigate the potential for an association between fish kills in the NC estuary system and the risk for adverse human-health effects. Impetus for the study was recent evidence suggesting that the estuarine dinoflagellate, *Pfiesteria piscicida*, may release a toxin(s) which kills fish and adversely affects human health. This report describes one component of the study in which visual system function was assessed. Study participants worked primarily in estuaries inhabited by *P. piscicida* or in off-shore waters thought not to contain *P. piscicida*. The potentially exposed estuary (N=22) and unexposed offshore (N=20) cohorts were well matched for age, gender, and education, but less well matched for occupation. Visual acuity did not differ between the cohorts, but visual contrast sensitivity (VCS), an indicator of visual pattern-detection ability for stimuli of various sizes, was significantly reduced in the estuary cohort relative to the offshore cohort. A further analysis which excluded participants having a history predictive of neuropsychological impairment also showed significantly reduced VCS in the estuary cohort (N=14) relative to the offshore cohort (N=10). Additional analyses indicated that differences between the cohorts in age, education, smoking, alcohol consumption, and total time spent on any water did not account for the difference in VCS. Finally, an analysis which excluded members of the estuary cohort who may not have had direct contact with an active fish kill, as well as offshore participants who may have had direct contact, also indicated that VCS was significantly lower in the estuary (N=17) than the offshore (N=17) cohort. The profile of VCS deficit across stimulus sizes resembled that seen in organic-solvent exposed workers, but an assessment of solvent and other neurotoxicant exposures did not indicate differences between the cohorts. These results suggest that factor(s) associated with the NC estuaries, including the possibility of exposure to *P. piscicida* toxin(s) at active fish kills, may impair visual system function.

## INTRODUCTION

The North Carolina Department of Health and Human Services, in collaboration with several universities and the U.S. Environmental Protection Agency, conducted a human-health study in the Fall of 1997 to investigate the potential association between fish kills in the North Carolina estuary system and human-health status. Impetus for the study was recent evidence suggesting that the estuarine dinoflagellate, *Pfiesteria piscicida*, may release a toxin(s) which kills fish (Burkholder et al., 1992, 1995) and adversely affects human health, particularly neurological function, in laboratory (Glasgow et al., 1995) and environmental (Morris et al., 1997; Bever et al., 1998; Golub et al., 1998; Grattan et al., 1998; Greenberg et al., 1998; Lowitt et al., 1998; Tracy et al., 1998) settings. A multi-component clinical evaluation was conducted to compare health status in: 1) two occupational cohorts, one with (the estuary cohort) and one without (the offshore cohort) potential for exposure to *P. piscicida* toxins; and 2) a case-control series in which the cases self-reported to the NC *Pfiesteria* Hot Line suspicions of having been affected by exposure to *P. piscicida* toxin(s). This report describes one component of the evaluation in which visual system function was assessed.

### Visual System Tests

Three tests of visual function were administered to participants in the current study. Two of the tests, visual contrast sensitivity (VCS) and visual acuity, were previously recommended by a panel of neurotoxicologists (Anger et al., 1994) for inclusion in a battery of core tests being assembled by the Agency for Toxic Substances and Disease Registry's (ATSDR) for use in environmental health field studies. Both tests were included in batteries designed for detecting subtle neurotoxic effects in adults (ATSDR, 1995) and children (ATSDR, 1996).

VCS is a measure of the ability to detect visual patterns (Ginsberg, 1984; Ginsberg et al., 1984). Whereas standard tests of visual acuity measure the visual system's resolution limit for high contrast stimuli, a task critically dependent on the functional integrity of the eye's physiological optics system, VCS is primarily an indicator of neurological function in the visual pathways from the retina to the cortex (Bodis-Wollner et al., 1986). The VCS test measures the least amount (threshold) of luminance difference (contrast) between adjacent areas necessary for an observer to detect a visual pattern. Contrast (C) is defined as: $C = (L_{max} - L_{min}) / (L_{max} + L_{min})$ where $L_{max}$ and $L_{min}$ are the luminances of the brighter and darker areas, respectively. VCS is the inverse of contrast threshold. A simple card test, the Functional Acuity Contrast Test (F.A.C.T.), measures contrast sensitivity for five sizes (spatial frequencies) of light and dark bar patterns (sinusoidal gratings) because spatial vision is mediated by populations of neurons selectively tuned to different spatial frequency (Bodis-Wollner et al., 1986). If neurons subserving low spatial-frequency (larger bars) vision are functionally impaired but those underlying high spatial-frequency (smaller bars) vision are functionally normal, for example, then visual perception also will be impaired for low frequency patterns but normal for high frequency patterns.

A standard test of visual acuity was administered to participants because VCS deficits at high spatial frequency could result from either refractive error or neurological dysfunction. A deficit in VCS at high spatial frequency in the presence of normal visual acuity indicates neurological dysfunction (Bodis-Wollner et al., 1986). VCS at low-to-mid spatial frequencies is unaffected by moderate acuity deficits (Bodis-Wollner et al., 1986). It is important to note that normal visual acuity is often found in neurotoxicological studies which show a significant reduction in VCS (Mergler et al., 1991; Frenette et al., 1991; Broadwell et al., 1995; Hudnell et al., 1996a,b,c). This arises from the fact that the acuity test engages only those neurons selectively tuned to small stimuli, and the stimuli are always of extremely high contrast. The VCS test, on the other hand, assesses visual function across stimulus sizes and engages neurons sensitive to low contrast in determining the sensitivity of the system to visual patterns.

A color discrimination test was also administered to screen for congenital color blindness and color vision deficiencies because severe dyschromatopsia could impair performance on tests of cognitive function which use chromatic stimuli, such as some neuropsychological tests and tests in the Neurobehavioral Evaluation System 2 (NES2; Baker et al., 1985), which was used in the current study. In addition, the color discrimination test included a condition designed to detect the failure to perform at the level of one's ability due to malingering or low motivation. Together, these three tests provided a basis for assessing group differences in visual function.

**Rationale for Test Selection**

Tests of visual function were included in the current evaluation for two primary reasons. Visual function is a sensitive indicator of neurotoxicity (Boyes, 1994; Mergler, 1995) and an important determinant of performance on tests designed to assess motoric and cognitive functions (Hudnell et al., 1996c). Two studies of mixed volatile-organic compound exposure observed VCS deficits in microelectronics-fabrication workers relative to unexposed control workers matched with exposed workers for age, gender, ethnicity, and education (Mergler et al., 1991; Bowler et al., 1991; Frenette et al., 1991; Broadwell et al., 1995; Hudnell et al., 1996a). Both studies observed a unique VCS profile across spatial frequencies; large VCS deficits were observed at mid-spatial frequencies with little or no deficit at higher and lower spatial frequencies. Although the magnitude of the VCS deficits was about 20%, severity was at a sub-clinical level; subjects had not been diagnosed with visual anomalies and generally attributed the reduction to normal ageing. Yet the significance of these deficits is striking in that participants of both studies had received little or no exposure for over a year prior to testing, suggesting that the deficits were permanent or long lasting. A study of patients previously diagnosed with organic-solvent-induced, chronic-toxic encephalopathy found a virtually identical pattern of VCS loss in the absence of recent exposure (Donoghue et al., 1995). Among currently exposed styrene workers, VCS reductions at the mid-spatial frequencies were significantly and inversely associated with end-of-shift urinary mandelic acid (a styrene metabolite) concentration (Campagna et al., 1995). Very recent data collected by EPA in cooperation with the New York State Department of Health suggested that people living in apartments above dry-cleaning facilities were at risk for alterations in VCS (Schreiber et al., 1998). Concentration of the dry cleaning solvent perchloroethylene (i.e.

tetrachloroethene) in apartment air was only about 1 ppm, but VCS was significantly reduced in 17 adults and children relative to matched-control subjects and showed the mid-spatial frequency deficit characteristic of solvent exposure. These results were consistent with a report of visual reaction-time and visual memory deficits in a German population with comparable environmental exposure to perchloroethylene (Altman et al., 1995). Analyses of the influence of VCS on results from visual reaction-time and visual memory tests (see below) suggested that VCS deficits may have been at least partially responsible for the reaction-time and visual memory deficits. Similar effects were observed in cohorts occupationally exposed to perchloroethylene (Seeber, 1989; Echeverria et al., 1995), and a follow-up study of previously exposed workers reported further decline in sensory and motoric, but not cognitive, functions after a mean exposure-free duration of 5.9 years (Lindstrom et al., 1982). The consistency of results from these studies, in conjunction with animal studies (Merigan et al., 1988; Boyes, 1994), suggest that a variety of organic solvents at relatively low exposure levels may act on a common mechanism to degrade mid-spatial frequency pattern vision.

Neurotoxicant induced deficits in VCS are not limited to solvent exposures. Visual-pattern evoked potentials (Hudnell et al., 1990a), a method for assessing pattern vision in animals and humans (Hudnell et al., 1990b; Hudnell and Boyes, 1991; Benignus et al., 1991), collected from rats indicated that acute exposures to several classes of pesticides, metals, and other compounds degrade pattern perception (reviewed in Boyes, 1992). Evidence is also mounting that some heavy metals may induce VCS deficits in humans. Measurements of VCS in children exposed to the combustion products of soft-brown coal in the Czech Republic revealed a pattern of low-to-mid spatial frequency loss. A significant association with methyl mercury body burden was observed (Hudnell et al., 1996b). Other studies have shown associations between VCS loss and methyl mercury (Mukuno et al., 1981; Lebel et al., 1996) and inorganic mercury exposures in adults (Cavalleri et al., 1995) and children (Altman et al., 1998). The VCS spatial-frequency profiles observed in the mercury exposed populations showed no evidence of the mid-spatial frequency selective deficit seen in solvent-exposed cohorts. These results are consistent with observations of methyl mercury-induced reductions of VCS in monkeys (Rice and Gilbert, 1982, 1990; Merigan et al., 1983).

Clinical studies have demonstrated that the VCS test is sensitive to the neurological dysfunction associated with many diseases affecting the nervous system. Ocular diseases, such as glaucoma, which manifests a low spatial-frequency deficit (Atkin et al., 1980; Ross et al., 1985; Sample et al.., 1991), macular disease (Loshin and White, 1984; Greeves et al., 1988), retinitis pigmentosa (Gawande et al., 1989; Seiple et al., 1993; Alexander et al., 1992, 1995), Type 1 diabetes with little or no retinopathy (Sokol et al., 1985; Trick et al., 1988 Bangstad et al., 1994), and other conditions (Bodis-Wollner and Camisa, 1980; Regan and Neima, 1984), produce a variety of alterations in the VCS spatial-frequency profile. VCS deficits, as well as color discrimination deficits (Mergler et al., 1987), are commonly present prior to detectible pathology in the retina or optic nerve head, making this one of the earliest sign of disease (Regan, 1989). With damage more proximal to the visual cortex, VCS deficits have been observed in cases of optic-nerve neuropathy (Bodis-Wollner, 1983), optic-nerve compression (Kuppersmith et al., 1982), and

cerebral lesions (Bodis-Wollner and Diamond, 1976). Patients that have recovered from optic neuritis with normal visual acuity retain severe deficits in VCS (Fleishman et al., 1987). Neurodegenerative diseases that are not well known for their effects on vision also manifest VSC deficits. Multiple sclerosis patients display VCS deficits which are orientation specific, suggesting cortical rather than retinal or optic nerve damage (Camisa et al.,1981). A primarily low spatial-frequency VCS deficit is present in Parkinson's (Regan and Neima, 1984) and Alzheimer's (Sadun et al., 1987; Cronin-Golomb et al., 1991; Gilmore and Levy, 1991) patients, the latter of whom show an extent of cognitive impairment predicted by VCS scores (Cronin-Golomb et al., 1995). AIDS patients display marked color-vision and VCS deficiencies (Quiceno et al., 1992), and cystic fibrosis patients show a VCS deficit across spatial frequencies which is either secondary to a vitamin A deficiency (Leguire et al., 1991) or, more likely, a primary manifestation of cystic fibrosis since the deficit is seen in patients taking vitamin A supplements (Morkeberg et al., 1995). Micronutirent deficiencies, including the vitamin B complex, are associated with reversible VCS deficits, as seen in the "Cuban epidemic optic neuropathy" cases of the early 1990s (Sadun et al., 1994; Roman, 1994). These studies suggest that the perception of visual patterns, as indicated by VCS, may be an apical endpoint that is commonly altered by a variety of clinical conditions which affect neurophysiological structures or biochemical processes in the visual pathways from the retina to the cortex.

VCS deficits thought to be congenital are associated with learning disabilities in children. Earlier research indicated that low VCS was prevalent among children with reading disabilities (Lovegrove et al., 1980) and dyslexia (Lennerstrand and Ygge, 1992). Recent evidence suggests that VCS deficits greatest at mid-to-high spatial frequency may be widespread among children with various types of learning disabilities (Hudnell et al., 1996b). This same distortion of the contrast sensitivity function was subsequently observed in Down syndrome children (Courage et al., 1997). A similar pattern of mid-to-high spatial frequency VCS reduction was seen in monkeys treated with acrylamide monomer which caused severe degeneration of the parvocellular retinogeniculate pathway while sparing the magnocellular pathway (Merigan et al., 1985, 1989, 1991). These results suggest that dysfunction in the parvocellular pathway may underlie mid-to-high spatial frequency VCS deficits.

Therefore, VCS deficits are associated with many abnormal neurological conditions, making the VCS test well suited as a tool for neurological health screening. Variations in the pattern of VCS loss across spatial frequencies are often associated with particular diseases and neurotoxicants, which increases the power of the VCS to assist in differential diagnostics. The above mentioned studies have demonstrated the potential for the VCS test as an aid in the diagnosis of acquired and congenital clinical conditions, as well as a tool for detecting neurotoxicant-induced subclinical deficits.

VCS appears to be not only a sensitive indicator for the adverse-health effects of a broad range of neurotoxicants and clinical conditions, but also is an important factor in the interpretation of computerized-neurobehavioral test data. Computerized tests are designed to assess a number of specific cognitive functions, for example visual memory and attention. These tests involve small or

briefly presented visual stimuli, and test endpoints often incorporate measurements of response times which may be as short as 150 ms. VCS is known to strongly influence detection of stimuli and, in turn, response times (Felipe et al., 1993; reviewed in Hudnell et al., 1996c). Yet historically, measures of visual function have not been used in the assessment of cognitive functions as indicated by computerized-test performance. The analysis of several data sets from environmental-epidemiological studies indicated that VCS can account for up to 24% of the variance in scores from NES tests (Hudnell et al., 1996c). In our study of solvent-exposed microelectronics fabricators (Broadwell et al., 1995; Hudnell et al., 1996a), VCS accounted for 18% of the variance in the simple-reaction-time test and with 17% of the variance in a pattern-memory test (Hudnell et al., 1998). These proportions were based on analyses of data collected from the unexposed control subjects, rather than the exposed subjects, to avoid the potential for correlated deficits in visual and cognitive functions induced by neurotoxicant exposure. A model was developed to remove the influences of vision from the data of both control and exposed participants in order to better assess the effects of neurotoxicant exposure on cognitive functions. Along with complementary procedures for calibrating the luminance and contrast of test stimuli on video screens (Hudnell et al., 1996c), the analytical model helped to both more accurately attribute performance deficits to the visual or cognitive domains and to reveal group differences in cognitive performance which were obfuscated by random differences in VCS. This effort was extended to include assessment and statistical control of motoric influences, as indicated by finger-tapping performance, on computerized test performance (Hudnell el al., 1998).

### The Current Study

Visual system function was assessed in the current study to test the hypothesis that VCS is lower in populations exposed to *P. piscicida* neurotoxins that in unexposed populations. The performance of several groups in the current study on tests of visual acuity, VCS, and color discrimination is reported. Visual acuity was measured to obtain an indicator of the functional integrity of the eyes' physiological optics system. VCS was measured as an indicator of neurological function in the visual system. The Ishihara color discrimination plate test was included to quickly screen for malingering and motivation to perform at the level of one's ability, as well as to detect congenital color blindness and color deficiencies because these conditions could affect performance on tests using colored stimuli. However, the Ishihara test is insensitive to acquired dyschromatopsia relative to tests designed for this purpose, such as the Lanthony 15 Desaturated Hue test (Geller and Hudnell, 1997). Results are presented for four separate group analyses: 1) The full occupational estuary and offshore cohorts; 2) the occupational cohorts restricted to participants without histories which could explain neurological deficits (Savitz, 1998); 3) the occupational cohorts restricted to estuary participants who reported direct contact with an active fish kill and offshore participants who did not report direct contact; and 4) the *Pfiesteria* Hot-Line case-control series participants.

## METHODS

Study design, study participant selection, methods for assessing socio-demographic factors and health history, and the criteria used to exclude participants with potentially confounding factors were described in detail by Savitz (1998), but will be briefly reviewed here. The investigators were unaware of the study participants' group status during testing, thereby creating a single-blind clinical investigation.

**Study Design & Subject Selection**

Occupational Cohorts

The occupational estuary- and offshore-cohort study design called for the selection of people who worked full time on the waters in 1997. The potentially exposed cohort worked in the Neuse and Pamlico river estuaries, whereas the offshore participants worked near their residences on the Outer Banks between Ocrachoke Village and Hatteras Village. The estuary participants were primarily licensed gill fishers and crabbers, although four were male state employees, some of whom had worked on the estuaries for only a few months. Ultimately, 19 males and four females were included in the estuary group. The offshore participants were licensed, commercial fishers, who worked in boats thought to be comparable in size to those of the estuary fishers, plus four male and four female county or state employees. The offshore participants were individually matched with estuary participants for age, gender, education, and occupation with a few exceptions, such as matching the four estuary fisherwomen with county or state employees due to the lack of suitable control fisherwomen. The data from several participants were ultimately excluded from analysis due to participants' failure to give good effort during testing or being outside of the targeted age or education range (Savitz, 1998). The current analyses of the full occupational cohorts are based on data from 22 estuary and 20 offshore participants who met all qualification criteria (Savitz, 1998). One offshore, but no estuary, participant had diabetes, a disease which might alter performance on vision or other neurobehavioral tests.

A sub-group from the estuary and offshore cohorts was also identified for analytical purposes (Savitz, 1998). Restricted cohorts were constructed to eliminate participants who had mild neurological anomalies that might be accounted for by factors other than exposure. These factors included a "history of difficulty in school, serious past or current psychiatric symptoms, daily marijuana use, past or current other drug abuse, and medical conditions with potential cognitive effects", including diabetes (Savitz, 1998). The restricted cohorts were comprised of 14 estuary and 10 offshore participants from the full group.

Analyses were also performed on a second sub-group of the full occupational group to exclude estuary participants who did not report direct contact with an active fish kill and offshore participants who did report direct contact with an active fish kill. This exclusion criterion resulted in 17 participants in both the estuary and offshore cohorts.

Case-Control Series

Cases were recruited for the study from the approximately 100 callers to the NC toll-free *Pfiesteria* Hot Line established after the State upgraded a Fish Kill Precaution to a Health Advisory in September, 1997 (Savitz, 1998). State telephone operators administered a 6 page questionnaire on health symptoms and water contact to all callers. NC Division of Epidemiology staff subsequently administered a more detailed follow-up questionnaire to 65 of the callers. Ten-point scales were used to quantify both exposure and symptom questionnaire data such that a maximum total score of 20 points could be attained. The highest scoring individuals were recruited for the study in descending score order until 11 agreed to participate. Case scores ranged from 15-20 points. A pool of 11 control subjects individually matched to cases for age, gender, education, and occupation was also recruited. Two of the controls were identified by cases as friends willing to participate, whereas the remainder of the controls were county health department employees from Eastern North Carolina and the Piedmont (Savitz, 1998).

**Vision Tests**

All subjects who normally wore corrective lenses for near-point viewing were asked to wear them during vision testing. The visual acuity and VCS tests were administered monocularly to each eye; an eye occluder was held over one eye while the other eye was tested. The Ishihara congenital color-blindness screen was administered binocularly. All vision tests were administered under illumination from a "daylight" illuminator (fluorescent source with a correlated color temperature of approximately = 6500° K; color rendering index > 90; intensity = 1150 lux; luminance approximately 70 foot-lamberts) in a clinical unit at East Carolina University Medical School which had normal background lighting. A light meter was used to insure that luminance remained constant throughout the test sessions. A face rest, placed just under the cheek bones and connected by a calibrated rod to a card holder on the distal end, was used to position the acuity and VCS test cards at a constant distance from the eyes (acuity - 36 cm; contrast sensitivity - 46 cm).

**Near Visual Acuity.** The acuity test card (Rosenbaum Pocket Vision Screener; Grass Instrument Co., Quincy, MA) contained 10 rows of numbers in which the size of the numbers progressed from a larger angular subtense in the top row to a smaller angular subtense in the bottom row. Participants were asked to first read the numbers in a middle row. Testing proceeded to the next lower row if all numbers were correctly identified or to the next higher row if an error occurred. The Snellen distance equivalent of the row with the smallest numbers which were all correctly identified was recorded as the visual acuity score. Approximately 2 minutes were required to test both eyes and explain the test results to the participants.

**Contrast Sensitivity.** The contrast sensitivity test card (Functional Acuity Contrast Test, F.A.C.T. 101; Stereo Optical Co., Chicago, IL) contained a matrix (5 x 9) of circles filled with sinusoidal gratings (dark and light bars). Spatial frequency (1.5, 3, 6, 12 and 18 cycles/deg) increased from top to bottom, and contrast decreased from left to right in steps of approximately 0.15 log units. The grating bars were oriented either vertically, or tilted 15 degrees to the left or right. As the investigator called out each circle from left to right, row by row, subjects responded

by saying either vertical, left, right or blank. Participants were encouraged to name an orientation if they had any indication that the bars could be seen. Participants were also asked to point in the direction to which the top of the grating was tilted if they felt any difficulty in verbalizing the orientation. The contrast of the last test patch correctly identified on each row was recorded as the contrast sensitivity score for that row (spatial frequency). The procedure was repeated for each row in descending order. Scores were recorded on a graph showing the normative range (90th percentile confidence interval). Approximately 6 minutes were required to test each eye separately and explain the results to the participants.

**Color Discrimination.** The short version (6 plates) of the Ishihara test (Ishihara's Tests for Colour-Blindness, 38 plates edition, 1993, Kanehara & Co., Ltd. Tokyo, Japan) was used to screen individuals for congenital dyschromatopsia. Four plates contained multicolored patches in which a number could be seen by participants with congenital trichromatic vision. One plate contained a number that could not be seen by participants with normal, trichromatic color vision; only participants with a red-green deficiency, either protanomaly or deuteranomaly, could identify the number. Another plate contained a number which could be seen by dyschromatopics since it was defined by luminance, rather than color, gradients. This plate served as a control for low motivation or malingering. All plates were individually displayed for binocular viewing and participants were informed of the results in about 1 minute.

**Vision Test Exclusion Criteria & Statistical Analyses.**

The units of analysis for the visual-acuity and contrast-sensitivity tests were the mean scores of the participant's two eyes for each endpoint, with one exception. The data from an eye was excluded from analysis if the visual acuity score was poorer than the Snellen Distance Equivalent of 20:70 in order to avoid confounding of the VCS results by excessive optical-refraction error. In cases where a participant had only one qualifying eye, the score from that eye was the unit of analysis. This visual acuity criterion for inclusion in data analysis did not result in the loss of any data among the occupational cohorts. However, data from four eyes in two *Pfiesteria* Hot-Line cases and four eyes in three Hot-Line controls were excluded due to the criterion. All participants in the occupational and *Pfiesteria* Hot-Line groups identified the Ishihara plate number defined by luminance, rather than color, gradients. Therefore, no additional exclusions were required due to low motivation to perform the tests or malingering. As mentioned above, several participants were previously removed from the population due to failure to meet the criteria described by Savitz (1998). Based on these results, the sample sizes remained at 22 estuary and 20 offshore participants in the unrestricted occupational group, 14 estuary and 10 controls in the group restricted for confounding factors, and 17 in both cohorts adjusted for fish kill exposure. The *Pfiesteria* Hot-Line group was reduced to nine cases and 10 controls.

Visual Acuity

Two-tailed Student t-tests with an $\alpha = 0.05$ were performed in all analyses of visual acuity, using each participant's mean visual acuity score, to determine if scores differed significantly between

cohorts.

Visual Contrast Sensitivity

The VCS data were analyzed using multivariate analyses of variance (MANOVA, with the Wilks' lambda statistic) procedures suitable for repeated measures with an $\alpha = 0.05$. The factors in the model were group, spatial frequency, and their interaction term. A factor for eye was not required since the analysis units were the mean scores for the two eyes at each spatial frequency, with the exception noted above concerning data from excluded eyes. A factor for gender was not included since no gender differences in susceptibility to *P. piscicida*-induced effects had been indicated and the groups were matched for gender. Results which showed a significant group-by-spatial frequency interaction were further analyzed in step-down, two-tailed Student t- tests ($\alpha = 0.05$), the equivalent of a univariate ANOVA, to determine which spatial frequencies accounted for the overall effect.

Additional analyses used multivariate linear-regression techniques to assess relationships between VCS, group assignment, and the covariates of age, education, smoking, alcohol consumption, and total time spent on any water. The model initially included each of these factors. A backward elimination technique was use to remove covariates and interaction terms which did not appear to explain any of the variance in VCS. Interaction terms and then covariates which had a p-value >0.015 were eliminated one at a time from the model if their removal did not alter the ratio of the group estimate and the standard error of the estimate. This approach allowed an assessment of the ability of group assignment to predict VCS while taking into account even small between-group differences in the covariates which might influence VCS. In addition, chi-square tests were used to assess the significance of group differences in several categorical variables.

Similar multivariate linear-regression analysis techniques were used in exploratory analyses (Muller et al., 1984) to evaluate the potential for several measures to serve as surrogates of exposure in dose-response assessments.

Color Blindness

Statistical anlayses were not performed on data from Ishihara's Tests for Colour-Blindness. Rather, each participant's data were examined using standard methods (Ishihara, 1993) to determine the presence or absence of congenital color blindness and color-discrimination deficiency.

## RESULTS

### Occupational Cohorts: Analyses of Group Differences

According to Savitz (1998), the estuary and offshore occupational cohorts were well matched for

age (mean estuary = 41.4; offshore = 42.9 years), education (mean estuary = 13.2; offshore = 13.8 years), and gender (mean estuary male = 78.3%, female = 21.7%; mean offshore male 81.8%, female = 18.2%). The matching of the estuary and offshore cohorts for occupation, based on job title, only attained a level of 52.2%. Whereas commercial fishers or crabbers comprised 73.9% of the estuary cohort, only 40.9% of the offshore participants shared these job titles. The lack of better matching for occupation was due in part to the inability to successfully recruit offshore female fishers or crabbers, which led to the alternative strategy of recruiting four female government employees for the offshore cohort.

The estuary and offshore participants in the full occupational group were not significantly different in visual acuity (Table 1). However, as shown in Figure 1, mean VCS was lower in the estuary than in the offshore participants at all five spatial frequencies. Statistical analyses indicated that the group factor and the group-by-spatial frequency interaction term were significantly different (Table 1). Significant VCS reductions in the estuary participants at the middle and next highest spatial frequencies, 6 and 12 cycles/degree (Table 1), were primarily responsible for the overall difference.

Since about half of the participants in the two occupational cohorts were identified as having historical factors which could influence neurobehavioral test outcomes (Savitz, 1998), the analyses of the vision data were repeated on the cohorts restricted to only those participants for whom potentially confounding medical, life-style, or educational factors were not identified. As in the full occupational group, the estuary and offshore restricted cohorts showed no statistically significant difference in visual acuity (Table 2). Yet, group differences in mean VCS were greater at each spatial frequency than in the full group due to slightly improved scores in the offshore participants (Figure 2). Both the group factor and the group-by-spatial frequency interaction term were significant (Table 2). Further analyses indicated that the significant difference between cohorts at the middle spatial frequency, 6 cycles per degree, primarily accounted for the overall difference (Table 2).

Due to the consideration that age, education, smoking, alcohol consumption, or total time spent on any body of water (an indicator of bright sunlight exposure, Rosenthal et al., 1991) might affect VCS and, therefore, that even small differences between the cohorts in these factors might account for the lower VCS scores in the estuary cohort, multivariate linear regression analyses were performed on the data sets from both the full and restricted occupational cohorts. The analyses sought to determine the ability of these variables plus group membership (estuary versus offshore) to predict VCS. Only VCS scores at the mid-spatial frequency, (6 cycles per degree, VCS-6), were used in the analyses because this variable showed the largest difference between the cohorts. The results for the full occupational group, shown in Table 3, indicated that group membership was the most significant predictor of VCS-6, although smoking and total time spent on any water appeared to account for some of the variance in VCS-6. In addition, there was a significant interaction between group and age. Multivariate linear regression analyses conducted on each cohort separately revealed a trend of decreasing VCS-6 with age in the offshore cohort, but not in the estuary cohort (Table 3). Similar results were seen in the analyses of the data from

the restricted occupational cohort (Table 3).

Since the VCS difference between cohorts was primarily due to reduced sensitivity at the middle spatial frequencies, as seen in solvent-exposed groups (Mergler et al., 1991; Bowler et al., 1991; Frenette et al., 1991; Broadwell et al., 1995; Hudnell et al., 1996a), the exposure-questionnaire data were examined to determine if the cohorts differed in occupational exposures to solvents or other potential neurotoxicants. The questionnaire design did not allow quantification of non-occupational exposures to potential neurotoxicants. As shown in Table 4, solvent exposures were commonly reported by members of both cohorts, but both the frequency of reports and the total number of years in which these exposures occurred were slightly greater in the offshore than estuary cohort. In addition, few participants in either cohort reported occupational exposures to mercury, lead, or pesticides. Although more participants reported exposures to other metals and fumes, these exposures were more frequent among offshore than estuary participants.

The exposure questionnaire data indicated that five members of the estuary cohort may not have had direct contact with an active fish kill, although they reported contact with some dead fish, and that three members of the offshore cohort may have been at the site of an active fish kill. Therefore, these participants were excluded from the full group in an analysis of VCS differences between the estuary (N=17) and offshore (N=17) cohorts. Mean VCS at each spatial frequency was higher in the offshore group than in the estuary group. The significance tests indicated that, although the group factor no longer showed a significant difference ($F(1,32)=2.95$, $p=0.096$), the difference in the group-by-spatial frequency interaction term remained significant ($F(4,29)=3.32$, $p=0.023$). Analyses of VCS at each spatial frequency again indicated that the overall difference between cohorts was largely due to a significance difference at the middle spatial frequency ($t=2.34$, $p=0.026$). The difference at other spatial frequencies was not significant.

The results of the color vision screening indicated that the estuary cohort included 1 (4.5%) congenitally colorblind, 11 normal (50%), and 10 (45.5%) color-deficient participants. The offshore cohort included no colorblind, 10 normal (50%), and 10 (50%) color-deficient participants.

## Occupational Cohorts: Exploratory Analyses of Potential *Pfiesteria*-Exposure Indicators

Exploratory analyses (Muller et al., 1984) of dose-response relationships were conducted using three variables constructed from the questionnaire data to assess the potential for the variables to serve as quantitative, although surrogate, indicators of exposure to *P. piscicida*. The analyses used multivariate linear regression techniques with the dependent variable, VCS-6, and the covariates of age, education, smoking, alcohol consumption, total time spent on any body of water, and each of the potential exposure indicators in separate analyses. The first potential exposure indicator was total time spent in estuary waters (TEW). The data from all members of the estuary cohort in the full occupational group and the three members of the offshore cohort who reported spending some time in estuary waters were included in the analysis. The regression

coefficient for VCS-6 and TEW, while in the direction of decreasing VCS-6 with increasing TEW, was not significant (p=0.20). A similar analysis, which omitted the covariate for total time spent on any body of water, and corresponding analyses on data from the restricted occupational group also failed to show a significant relationship between VCS-6 and TEW.

The other two potential exposure indicators were participant-reported number of contacts with fish kills (NFK) and the total number of hours spent at fish kills (HFK). Among the estuary cohort members, HFK showed a significant interaction with years of education in the multivariate linear regression with VCS-6 (estimate=-0.28, standard error=0.09, p=0.009), whereas NFK showed a trend in the same direction (estimate=-6.2, standard error=2.9, p=0.051). Of the 22 members in the estuary cohort, 10 participants had 12 or fewer years of education and 12 participants had 13 or more years of education. To address the VCS and education interaction, simple regression analyses of VCS-6 with HFK were performed separately on the two education groups. HFK and VCS-6 were significantly related in the more highly educated group (estimate=-0.28, standard error=0.12, p=0.046) but not in the less educated group (estimate=0.10, standard error=0.19, p=0.618). Participants in the more highly educated group were more likely to have contacted a fish kill than those in the less educated group (correlation chi square, p=0.011), and six of the seven participants who reported contact with 3 or more fish kills were in the more highly educated group.

### Case-Control Series

The mean age of the *Pfiesteria* Hot-Line cases was 43.7 years and they averaged 12.5 years of education. Males and females comprised 72.7% and 27.3% of the group, respectively (Savitz, 1998). Although initial screening indicated that all cases had direct contact with fish kills, only six of the 10 cases confirmed contact during the clinical examination (Savitz, 1998). Demographic characteristics of the control group were not described (Savitz, 1998).

Visual acuity was slightly better in the *Pfiesteria* Hot-Line control participants than in the cases, although the difference was not statistically significant (Table 4). However, the control group scored lower in VCS than the cases at each spatial frequency (Figure 3), although neither the group factor nor the interaction term showed a statistically significant difference between the cases and controls (Table 4).

The results of the color vision screening indicated that the cases included no colorblind, 2 normal (22.2%), and 7 (77.8%) color-deficient participants. The controls included 1 (10%) colorblind, 7 normal (70%), and 2 (20%) color-deficient participants.

## DISCUSSION

### Occupational Cohorts: Confirmatory Analyses

Several analyses indicated that the estuary cohort had a mid-spatial frequency reduction in VCS relative to the offshore cohort. First, in the full occupational cohort, the significant difference in the MANOVA group factor indicated that VCS averaged across spatial frequencies was lower in the estuary cohort than in the demographically-matched offshore cohort. The significance of the group-by-spatial frequency interaction term indicated that the two cohorts' VCS profiles across spatial frequencies were not parallel. It can be seen in Figure 1 that peak sensitivity in the offshore group was at 6 cycles per degree of visual arc, as seen in other unexposed populations (Hudnell et al., 1996b,c). In the estuary group, however, no clear peak in sensitivity was apparent; sensitivity was approximately equal at 3 and 6 cycles per degree. Analyses of group differences at each spatial frequency indicated that VCS was significantly reduced in the estuary cohort at 6 and 12 cycles per degree relative to the offshore cohort.

Second, an assessment of medical history, life-style factors, and educational history indicated that these factors might explain deficits in neurological function in about half of the participants in both cohorts (Savitz, 1998). Therefore, group differences in VCS were reassessed after restricting the cohorts to participants free of these potentially confounding factors. As can be seen by comparing Figures 1 and 2, the restriction had little effect on the VCS profile of the estuary cohort, but led to some improvement in the VCS profile of the offshore cohort. The difference in VCS between cohorts and the group-by-spatial frequency interaction term were significantly different in the restricted group. Analyses at each spatial frequency indicated that the difference in VCS at 6 cycles per degree was significant, whereas that at 12 cycles per degree only approached significance.

Third, multivariate linear-regression analyses with backward elimination of interaction terms and variables unrelated to VCS were performed to assess the potential for differences between the cohorts in age, education, smoking, alcohol consumption, and total time spent on any water to account for the group difference in VCS at the mid-spatial frequency (VCS-6). Age (Green and Madden, 1987), smoking (West et al., 1989, 1995), alcohol consumption (Roquelaure et al., 1995), and sunlight exposure (Taylor, 1995; Javitt and Taylor, 1995; Schein et al., 1994; Taylor et al., 1993; Werner et al., 1990; Taylor et al., 1990; Taylor et al., 1989) have been reported to affect either the optical properties or neurological function of the visual system. Both smoking and total time spent on any water appeared to account for some of the variance in VCS-6, and a relationship between VCS and age was observed in the offshore, but not the estuary, cohort. Education and alcohol consumption did not appear to influence VCS-6 in the full or restricted occupational cohorts. However, a strong difference in VCS-6 between the estuary and offshore groups remained after taking into account the five potential covariates.

Fourth, an analysis of questionnaire data on occupational exposures to potential neurotoxicants did not indicate greater neurotoxicant exposures in the estuary cohort, suggesting that such exposures were not likely the cause of VCS differences between the estuary and offshore cohorts. Fifth, another restricted group was created from the full occupational group because the exposure questionnaire data indicated that 5 participants in the estuary cohort may not have had direct contact with an active fish kill, whereas 3 members of the offshore cohort may have had contact.

Analyses indicated that, although the group factor for VCS only approached significance, the interaction term and the group difference at 6 cycles per degree remained significant. However, this analysis failed to show a larger difference than that seen in the full group as might have been expected if direct contact with *P. piscicida*-induced fish kills was the causative agent, although this qualification is somewhat mitigated by the fact that fish kills contacted by the offshore participants may have been caused by factors other than *P. piscicida*.. The crude assessment of color discrimination ability provided by the Ishihara test did not indicate differences in dyschromatopsia between the full occupational cohorts. However, since the VCS profile resembled that seen in studies of solvent exposure, and solvent exposures also cause dyschromatopsia (reviewed in Geller and Hudnell, 1997), future studies of *P. piscicida* effects in humans should use color discrimination tests more sensitive to acquired dyschromatopsia such as the Lanthony 15 Desaturated Hue test (Geller and Hudnell, 1997).

**Conclusions and Possible Implications.** Taken together, analyses of the VCS data confirm the hypothesis that some factor(s), other than several demographic variables, medical history, life-style factors, or known neurotoxicant exposures, is associated with lower visual system function in the estuary cohort than in the offshore cohort. However, the nature of the association remains unclear. As in all studies involving group comparisons, it is possible that the difference observed between the cohorts resulted from chance. The selection of cohort members from the entire populations of offshore and estuary fishers and crabbers could have resulted in the inclusion of participants with lower VCS in the estuary cohort and participants with higher VCS in the offshore cohort simply by chance, even if there is no difference in VCS between the entire populations. However, if the entire populations do differ in VCS, there are at least three primary hypotheses that may explain the difference. First, since all estuary participants lived on or near the mainland and all offshore participants lived on the outer banks, the group difference in VCS could be due to an unknown factor(s) (e.g. neurotoxicant exposure, population genetics) which differs between these geographical areas. Second, a factor(s) associated with estuary waters other than *P. piscicida* (e.g. other neurotoxin or neurotoxicant exposure) could have caused the group difference in VCS. Third, exposure to *P. piscicida* neurotoxins in the estuaries could have caused the deficit in visual function. If the VCS deficit is attributable to contact with *P. piscicida*-induced fish kills, the lack of recent exposure (Savitz, 1998) would suggest that the effect is permanent or long lasting, as previously seen with solvent-induced VCS deficits (Mergler et al., 1991; Bowler et al., 1991; Frenette et al., 1991; Broadwell et al., 1995; Hudnell et al., 1996). Alternatively, the VCS deficit could be more readily reversible, but caused by unknown, non-exposure related factor(s) or by some other, perhaps more continuous, exposure factor(s) associated with the estuaries or the geographical area. Given the multiple possible explanations and the study limitations discussed below, each of these hypotheses should be viewed as tentative.

The average contrast of the mid-spatial frequency VCS test stimulus required for the participants to detect the stimulus was about 30% higher in the estuary cohort than in the offshore cohort. The functional impact of this difference in perceptual threshold, the inverse of contrast sensitivity, is difficult to estimate, although a number of studies have assessed the relationship between VCS and various functional abilities. It is well recognized that the speed of performance on behavioral

tasks, as indicated by reaction times, is highly dependent on VCS (Felipe et al., 1993; Hudnell et al., 1996c). Since delays in the detection of visual stimuli due to VCS deficits cause a delay in the production of appropriate responses, VCS scores have been proposed for use as a predictor of automobile-accident risk in elderly drivers (Shinar and Schieber, 1991). In the elderly, VCS is strongly associated with postural stability (Lord et al., 1991) and the frequency of falling (Lord et al., 1994). VCS scores helped to correctly categorize individuals into multiple faller and non-multiple faller categories with 75% accuracy (Lord et al., 1994). Furthermore, other studies have associated VCS with reading performance (Carmean and Regeth, 1990) and athletic ability (Kulka et al., 1989, 1993, 1996; Love and Kulka, 1992; Melcher and Lund, 1992). This evidence suggests that VCS deficits may result in decreased productivity and a lessening of the quality of life due to slowed performance and an increased risk for accidents. Although VCS deficits may be predictive of cognitive impairment in neurodegenerative disease (Cronin-Golomb et al., 1995), no evidence suggests that the estuary cohort participants are at increased risk for any disease.

**Occupational Cohorts: Exploratory Analyses**

The exploratory analyses, classified as such to reduce the probability of obtaining false-positive results due to multiple comparisons (Muller et al., 1984), attempted to identify surrogate measures of exposures in the estuary waters. No relationship between VCS-6 and total time spent on estuary waters was apparent. This result suggested that, if VCS was reduced in the estuary cohort due to exposures encountered while on the estuaries, the exposure was not likely to have been continuously ongoing, but rather was likely to have been periodic as would be expected if the causative exposure was *P. piscicida* neurotoxin(s) released during fish kills. A significant interaction between total hours spent at fish kills (HFK) and educations was observed in a multivariate linear regression with VCS-6 in the estuary cohort. Simple linear regressions of VCS-6 with HFK were performed on roughly even numbers of participants who did and did not have at least some college education. A significant correlation was observed in the more highly educated group but not in the less educated group. However, the more highly educated group had significantly more contacts with fish kills than the less educated group. A stronger association of VCS with HFK would be expected among more highly exposed than lesser exposed participants if an above-threshold level of exposure was required to produce the VCS deficit. This result suggested that future studies may be able to associate health effects with exposure by obtaining detailed information from study participants on contact with fish kills.

**Related Research**

The results from the assessment of visual system function were consistent with the outcomes of the neurological examination (Savitz, 1998) in which a trend towards a difference between the occupational cohorts in sensory abnormalities was observed. Although the difference was not significant, overall sensory abnormalities were noted in 41% of the full cohort of estuary participants versus 20% of the offshore cohort members (risk ratio=2.0, 95% CI=0.8-5.6). Sensory abnormality ratings in the group restricted for potentially explanatory histories were 43% in the estuary cohort and 18% in the offshore cohort (risk ratio=2.4, 95% CI=0.3-19.6), but this

difference was not significant. The only other category in the neurological examination showing notable, but nonsignificant, differences between groups was overall peripheral neuropathy. The frequencies of peripheral neuropathy in the full group were 35% and 18% in the estuary and offshore cohorts, respectively. Comparable figures in the group restricted for potentially explanatory histories were 36% in the estuary cohort and 9% in the offshore cohort. Little or no difference between cohorts in either the full or restricted group was observed for cognitive or motoric function, behavioral disturbances, neurotoxic complex staging, or affective status (Savitz, 1998).

Scientists exposed *to P. piscicida* toxins in the laboratory have reported ocular irritation, as well as blurred vision which persisted for hours to days (Glasgow et al., 1995), although the neurological signs and symptoms given greater prominence included narcosis, short-term memory loss, spatial disorientation, peripheral sensory disturbance, and emotional lability (Glasgow et al., 1995). Clinical evaluations of humans recently exposured to a *Pfiesteria*-induced fish kill were conducted in Maryland during the summer of 1997 (Morris et al., 1997; Bever et al., 1998; Golub et al., 1998; Grattan et al., 1998; Greenberg et al., 1998; Lowitt et al., 1998; Tracy et al., 1998). VCS and other neurobehavioral tests of visual system function were not administered in the Maryland study. Neurological assessment in the Maryland study focused primarily on neurocognitive functions. Cognitive function was also the focus of the only study reported to date which investigated the effects of *P. piscicida* exposure in an animal model (Levin et al., 1997). Water containing *P. piscicida* was collected from an aquarium in which a fish kill had been induced, and single subcutaneous injections were given to rats. The exposed rats and control rats, which received *P. piscicida*-free water injections, were repeatedly tested with a radial-arm maze learning task. Choice accuracy was significantly reduced in the exposed rats relative to controls for up to 10 weeks. Subsequent tests showed no performance differences between groups. Rats trained on the task prior to exposure showed no decrement in performance relative to control rats, suggesting that *P. piscicida* exposure induced a learning, rather than a memory, deficit. However, no assessment of visual function in the rats was performed. Since performance in radial-arm maze tasks is heavily dependent on visual cues (e.g. Zoldek and Roberts, 1978), the learning delay seen in the exposed rats may have been due to a reduction in visual system function. Humans or animals with low vision may require a longer period of time to learn a visually oriented task than those with normal vision, but then perform at a level comparable to those with normal vision after finally mastering the task.

**Case-Control Series**

Analyses of data from the *Pfiesteria* Hot Line case-control series indicated that these groups were not significantly different in VCS. A notable feature of the data was that the VCS profile of the control participants (Figure 3), who scored lower at each spatial frequency than the cases, was well below that of the offshore cohorts in the occupational group (Figures 1, 2). The incidence of color-vision deficiency, on the other hand, was low in the controls relative to the cases and the offshore cohorts in the occupational group. However, due to the relative insensitivity of the Ishihara test to acquired dyschromatopsia and its inability to distinguish congenital from acquired

color deficiency (Geller and Hudnell, 1997), the color discrimination data provided only weak support for the position that the cases and controls differ in acquired dyschromatopsia. Overall, the VCS and color discrimination data do not indicate that visual function in the *Pfiesteria* Hot-Line cases was affected by exposure to NC estuaries.

**Study Strengths and Weaknesses**

As noted by Savitz (1998), strengths of the current study included: 1) the use of objective criteria in the subject-selection process; 2) matching of the estuary and offshore cohort participants for age, gender, and education, with less complete matching for occupation, and; 3) masking of the investigators with respect to the exposure status of the participants. However, a number of factors preclude drawing definitive conclusions concerning the cause of the VCS deficits seen in the estuary cohort. Study limitations noted by Savitz (1998) included: 1) the possibility that the prevalence of many factors with potential to affect neurological function, and thereby confound interpretation of the study results, may not have been well balanced between cohorts; 2) uncertainty about the participants' exposure to *P. piscicida* toxin(s), and; 3) a lack of any participants with very recent fish kill contact. Uncertainties concerning potentially confounding factors are a major source of concern in drawing inferences from the study results. For example, data obtained on chronic alcohol consumption, which can reduce VCS (Roquelaure et al., 1995), the use of neuroactive prescription drugs, and avocational or environmental exposures to neurotoxicants may have been inadequate to insure that these factors did not account for the VCS differences between cohorts. In addition, no data were collected on other potentially confounding factors such as dietary habits and the use of sunglasses or brimmed hats. The Cuban epidemic optic neuropathy episode demonstrated that deficiencies in micronutrients without overt malnutrition can induce reversible VCS deficits (Sadun et al., 1994, Roman, 1994). Chronic exposure to intense sunlight, particularly the ultra violet portion of the spectrum, can induce cataracts and other conditions involving optical aberrations (Taylor, 1995; Javitt and Taylor, 1995; Schein et al., 1994; Taylor et al., 1993; Taylor et al., 1989), which reduce VCS (Lasa et al., 1992; Drews-Bankiewicz et al., 1992; Burton et al., 1993). Sunlight exposure also has been proposed as an important determinant of retinal aging (Werner et al., 1990) and age-related macular degeneration (Taylor et al., 1990), and VCS declines with aging beyond about 40-50 years (Green and Madden, 1987). Less frequent use of brimmed hats and sunglasses by the estuary workers than the off-shore workers could have induced optical opacities or accelerated retinal aging which might account for the VCS difference between cohorts. Analyses of the VCS data only included eyes with a visual acuity of 20:70 or better. However, detection of the extremely high-contrast stimuli used in the visual acuity test may be less affected by the optical and neural effects of chronic sunlight exposure than is detection of the low-contrast VCS stimuli. Future studies could directly address the issue of ocular aberrations by performing slit-lamp examinations on the participants (Taylor, 1995). It is possible that differences between cohorts in these or other factors could have caused the difference in VCS.

Uncertainties in human-health risk assessments decrease as more information becomes available to quantitatively describe relationships between the components of a biologically-based dose-

response model (BBDR; e.g. Conolly and Andersen, 1991 Andersen et al., 1992), illustrated in Figure 4. In order to implicate (or exonerate) *P. piscicida* as the causative agent of the VCS difference between cohorts, more definitive data are needed on exposure - the dose of neurotoxin(s) applied to humans, the absorbed dose, and dose at the target site. In the current study, time spent on the water and the degree of potential *P. piscicida* exposure in the estuary cohort varied greatly. Some government employees in the estuary population had been employed for only five months at the time of the study and had very limited activity on the water, whereas some fishers and crabbers had more than 20 years of potential exposure. As a first approximation of the applied dose, the current study tried to identify the date, location, and time spent in direct contact (within 6 feet) with active fish kills. Yet uncertainties in the participants' abilities to recall and report this information, and the lack of evidence that *P. piscicida* was the cause of all reported fish kills, permitted only crude quantifications of surrogates for applied dose. These factors could be better assessed in a prospective study by collecting exposure data during an active fish kill. Identification of the *P. piscicida* toxin(s) would enable better quantification of applied and absorbed doses if methods were developed to measure the toxin(s) in water, air (or mist above the water), and in biological samples. More complete information on exposure dosage would permit dose-response assessments and better justify causative inferences between *P. piscicida* and effects such as reduced VCS.

**Conclusion and Research Needs**

The current study sought to provide initial evidence on the human health effects of exposure to *P. piscicida*-induced fish kills, and to identify experimental design factors which might improve future studies. The current study design allowed associations to be made between VCS reduction and inland residence with time spent on NC estuaries, relative to offshore residence with time spent on off-shore waters. Since the time spent working on estuary waters sometimes involved contact with fish kills thought to be induced by *P. piscicida* toxin(s), one of the possible causative agents is *P. piscicida*. However, due to uncertainties about exposure to *P. piscicida* toxin(s) and the confounding of residency area, estuary contact, and fish-kill contact, future research must verify the tentative association between exposure to *P. piscicida* neurotoxin(s) and VCS reduction. Verification of this relationship would indicate that the VCS test is a useful screening tool for detecting an early sign of *P. piscicida* exposure-induced effects on neurological function.

Two approaches might be taken in future studies to more strongly implicate (or exonerate) *P. piscicida* as the causative agent. First, a strong association between *P. piscicida* and health status could be attained using a prospective approach with health measurements taken before and after exposure to fish kills where environmental monitoring demonstrated the presence of *P. piscicida* and, if possible, *P. piscicida* toxin(s). This approach would also provide an opportunity to detect any rapidly-reversible effects, an element missing from the current study. However, if the participants in a prospective study were previously exposed to *P. piscicida* toxin(s), persistent exposure-induced effects present during the initial evaluation could lessen the probability that effects would be detected after a subsequent exposure. Second, a comparison of estuary workers who were and who were not at a recent fish kill where *P. piscicida* activity was documented

could eliminate any confounding differences between cohorts associated with residence area and estuary versus off-shore work. However, this approach is also subject to the limitation imposed by pre-existing, exposure-induced effects. The use of young, previously unexposed workers to obviate this problem might be inadequate because young workers may be less susceptible to *P. piscicida* toxin(s) than older workers due to greater neurological reserve or compensatory capacities. Susceptibility may increase through an interaction with the functional declines associated with ageing such that dysfunction is more likely to manifest in older individuals, as observed with the Parkinson-like motoric dysfunction associated with environmental manganese exposure (Mergler et al., 1998; Hudnell, 1998).

A more optimal approach might be formed by combining the best aspect of the current study's design, the single-blind group comparison, with a prospective, repeated-measures approach. Two large cohorts, one with and one without exposure potential, could be established and maintained long-term. Workers could be selected for the cohort with exposure potential who had not previously contacted an active fish kill. An extensive assessment could be undertaken during the selection process to screen potential participants for confounding factors and to individually match the exposed and control cohort participants for socio-demographic characteristics. Initial health assessments could evaluate the comparability of neurological function in matched-pairs of participants. Following subsequent exposure to a confirmed and monitored *P. piscicida*-induced fish kill, the matched-pairs could be reassessed to determine the health risk of exposure to *P. piscicida* toxin(s). Limitations to this approach are cost and the possibility that a widespread perception of risk associated with fish kill contact, or a NC ban on water activities during a fish kill, could result in none of the participants being exposed. In this case, a study could be designed to assess the potential for risk in shore-side residents at locations with and without a high probability for fish kills. In addition, further development of an animal BBDR model for studying the neurotoxic effects of *P. piscicida* toxin(s) (Levin et al., 1997) could improve human-health risk assessments for exposure to *P. piscicida* toxin(s) by investigating dose-response relationships and the mechanism(s) of toxicity.

## ACKNOWLEDGMENTS

This manuscript benefited greatly from information in the report on the study written by Dr. David Savitz, UNC School of Public Health, who also supplied materials needed to do the analyses reported in this manuscript. The assistance of Cristin Cahill-Harrell, US EPA, in collection of these data, and Dennis House and Judy Schmid, US EPA, in performing the statistical analyses, is gratefully acknowledged. Dr. Woodhall Stopford, Duke University Medical School, supplied suggestions for data analyses and data for two variables on time spent in estuary and other waters which enhanced the analyses. Reviews of earlier versions of the manuscript by Drs. William Boyes, Hugh Tilson, Robert MacPhail, and Vernon Benignus, US EPA, Dr. Woodhall Stopford, and anonymous reviewers led to substantial improvements in the manuscript. The clinical evaluations of study participants were made possible by the organizational skills of Dr. Marian Swinker, East Carolina University Medical School, and her staff. Finally, Dr. Stanley Music, NC Department of Health and Human Services, is credited for the insight and leadership which made the study a reality.

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**Table 1** **Full Occupational Group**

**Visual Acuity**

| Group | N | Snellen Distance Equivalent (20:X) | SEM | t-score | p-value |
|---|---|---|---|---|---|
| Offshore | 20 | 29.75 | ± 3.0 | 0.27 | 0.79 |
| Estuary | 22 | 30.80 | ± 2.3 | | |

**Visual Contrast Sensitivity**

| Source | Degrees of Freedom | F-score | p-value |
|---|---|---|---|
| Group | 1, 40 | 5.40[1] | 0.025 |
| Spatial Frequency (SF) | 4, 37 | 130.71[2] | <0.001 |
| Group x SF | 4, 37 | 5.04[2] | 0.002 |

[1] Univariate ANOVA [2] MANOVA (Wilks' lambda statistic)

**Visual Contrast Sensitivity by Spatial Frequency**

| Spatial Frequency (cpd) | Offshore Mean VCS | Offshore SEM | Estuary Mean VCS | Estuary SEM | t-score | p-value |
|---|---|---|---|---|---|---|
| 1.5 | 54 | ± 3.0 | 48 | ± 3.7 | 1.24 | 0.224 |
| 3 | 89 | ± 7.1 | 78 | ± 5.6 | 1.19 | 0.242 |
| 6 | 107 | ± 9.0 | 78 | ± 6.5 | 2.70 | 0.010 |
| 12 | 56 | ± 7.0 | 40 | ± 3.1 | 2.09 | 0.043 |
| 18 | 26 | ± 3.3 | 21 | ±2.5 | 1.38 | 0.174 |

**Table 2** **Restricted Occupational Group**

**Visual Acuity**

| Group | N | Snellen Distance Equivalent (20:X) | SEM | t-score | p-value |
|---|---|---|---|---|---|
| Offshore | 10 | 31.25 | ± 4.6 | 0.66 | 0.52 |
| Estuary | 14 | 27.9 | ± 2.7 | | |

**Visual Contrast Sensitivity**

| Source | Degrees of Freedom | F-score | p-value |
|---|---|---|---|
| Group | 1, 22 | 4.59[1] | 0.044 |
| Spatial Frequency (SF) | 4, 19 | 68.83[2] | <0.001 |
| Group x SF | 4, 19 | 3.14[2] | 0.038 |

[1] Univariate ANOVA [2] MANOVA (Wilks' lambda statistic)

**Visual Contrast Sensitivity by Spatial Frequency**

| Spatial Frequency (cpd) | Offshore Mean VCS | Offshore SEM | Estuary Mean VCS | Estuary SEM | t-score | p-value |
|---|---|---|---|---|---|---|
| 1.5 | 59 | ± 3.3 | 50 | ± 5.2 | 1.20 | 0.245 |
| 3 | 98 | ± 11.9 | 80 | ± 6.3 | 1.41 | 0.173 |
| 6 | 115 | ± 15.1 | 79 | ± 8.1 | 2.28 | 0.032 |
| 12 | 61 | ± 11.5 | 40 | ± 4.0 | 1.91 | 0.070 |
| 18 | 29 | ± 5.6 | 22 | ±3.4 | 1.14 | 0.265 |

**Table 3 Is the Relationship Between VCS at the Mid-Spatial Frequency and Group Affected by: Age, Education, Smoking, Alcohol, Total Time on Any Water or First Order Interactions?**

**Multiple Linear Regression Analyses With Backward Elimination[1]**

**Full Occupational Group**

| Parameter[1] | Estimate | Standard Error | p - Value |
|---|---|---|---|
| Intercept | 175.2 | 33.1 | <0.001 |
| Group | -118.5 | 44.1 | 0.011 |
| Age | -1.80 | 0.79 | 0.030 |
| Smoking | -0.53 | 0.32 | 0.104 |
| Total Time on Water | 0.008 | 0.004 | 0.039 |
| Group x Age | 2.15 | 1.04 | 0.047 |

[1] Parameters were eliminated from the model if $p > 0.15$ and removal of the parameter did not change the relationship of VCS at the mid-spatial frequency with group

**Regression of VCS-6 with Age By Cohort[2]**

| Cohort | Slope | Standard Error | p - value |
|---|---|---|---|
| Estuary (N=22) | 0.42 | 0.67 | 0.545 |
| Offshore (N=20) | -1.81 | 0.87 | 0.055 |

[2] The model also included smoking and total time on the water as predictors

**Restricted Occupational Group**

| Parameter[1] | Estimate | Standard Error | p - Value |
|---|---|---|---|
| Intercept | 287.1 | 52.9 | <0.001 |
| Group | -265.3 | 66.5 | 0.001 |
| Age | -4.60 | 1.30 | 0.003 |
| Smoking | -0.64 | 0.33 | 0.072 |
| Total Time on Water | 0.012 | 0.004 | 0.007 |
| Group x Age | 5.75 | 1.62 | 0.002 |

[1] Parameters were eliminated from the model if $p > 0.15$ and removal of the parameter did not change the relationship of VCS at the mid-spatial frequency with group

**Table 4** **Occupational Neurotoxicant Exposures in the Full Group**

| | Hg | Pb | Other Metals | Pesticides | Fumes | Solvents | Solvent Years |
|---|---|---|---|---|---|---|---|
| Estuary (N=22) | 1 | 1 | 5 | 2 | 6 | 10 | 130 |
| Offshore (N=20) | 2 | 2 | 8 | 1 | 8 | 11 | 136 |

**Table 5** **Hot Line Cases & Controls**

**Visual Acuity**

| Group | N | Snellen Distance Equivalent (20:X) | SEM | t-score | p-value |
|---|---|---|---|---|---|
| Controls | 10 | 25.80 | ± 3.4 | 0.54 | 0.55 |
| Cases | 9 | 28.90 | ± 4.7 | | |

**Visual Contrast Sensitivity**

| Source | Degrees of Freedom | F-score | p-value |
|---|---|---|---|
| Group | 1, 17 | 1.08[1] | 0.314 |
| Spatial Frequency (SF) | 4, 14 | 69.81[2] | <0.001 |
| Group x SF | 4, 14 | 1.29[2] | 0.319 |

[1] Univariate ANOVA [2] MANOVA (Wilks' lambda statistic)

## FIGURE LEGENDS

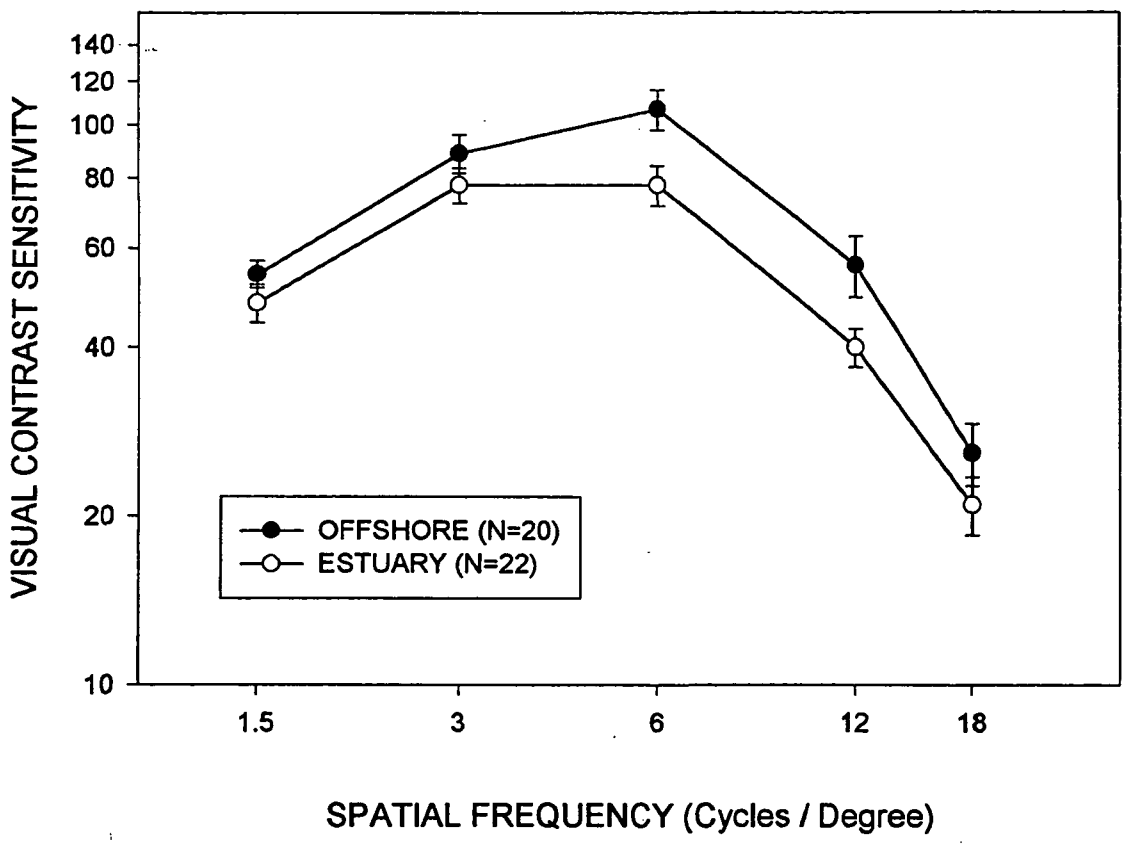
**Figure 1.** Visual contrast sensitivity (mean ± SEM) functions for the estuary and offshore cohorts in the full occupational group. MANOVA analyses indicated that the group factor and the group-by-spatial frequency interaction term were significantly different. Step-down tests indicated that the VCS scores of the estuary cohort at 6 and 12 cycles per degree of visual arc were significantly lower than that of the offshore cohort.

**Figure 2.** Visual contrast sensitivity (mean ± SEM) functions for the estuary and offshore cohorts in the occupational group restricted to include only participants free of potentially confounding factors. MANOVA analyses indicated that the group factor and the group-by-spatial frequency interaction term were significantly different. Step-down tests indicated that the VCS score of the estuary cohort at 6 cycles per degree of visual arc was significantly lower than that of the offshore cohort.

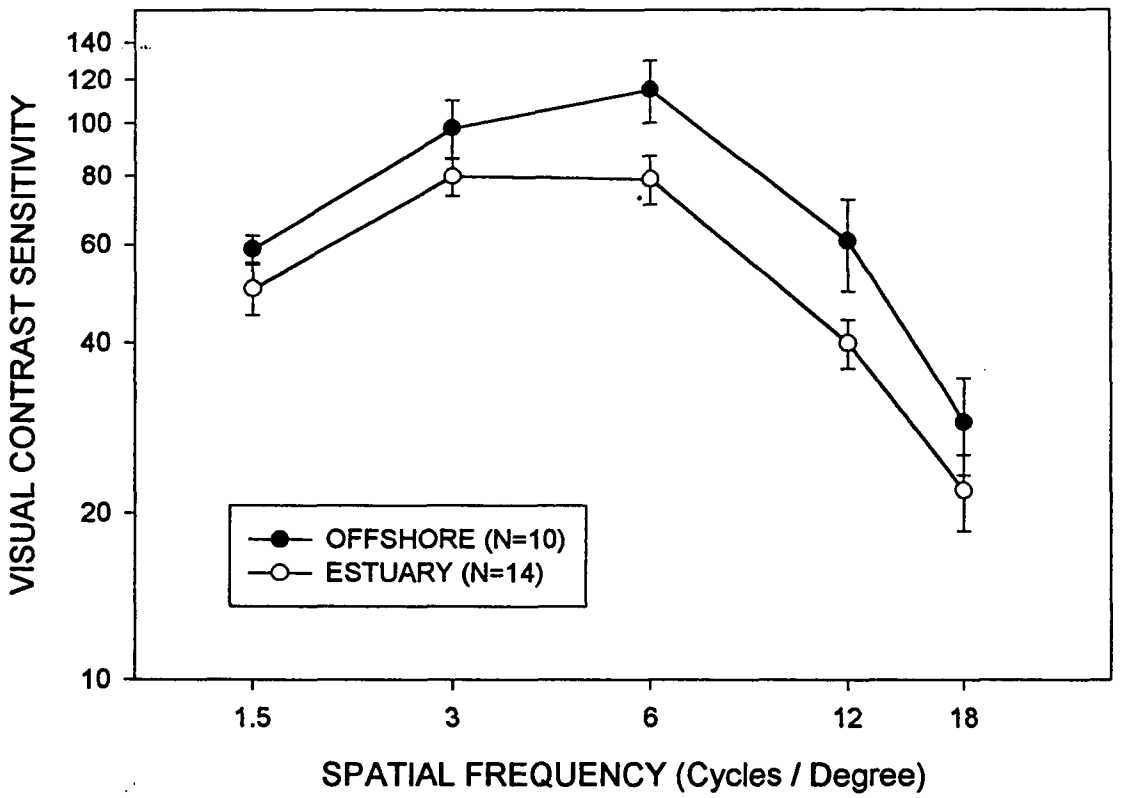
**Figure 3.** Visual contrast sensitivity (mean ± SEM) functions for the cases recruited from the NC Pfiesteria Hot-Line and matched-control participants. MANOVA analyses indicated that neither the group factor nor the group-by-spatial frequency interaction term were significantly different.

**Figure 4.** A Biologically-Based Dose-Response Model (BBDR) for the health effects of environmental toxin exposure in humans. The ultimate goal of the model is to mathematically describe relationships between each of the stages from sources to health outcome. More complete data on the dose of Pfiesteria toxin(s) applied to humans, as well as the absorbed dose, are needed to support the hypothesis of a causal link with the observed difference between cohorts in VCS.

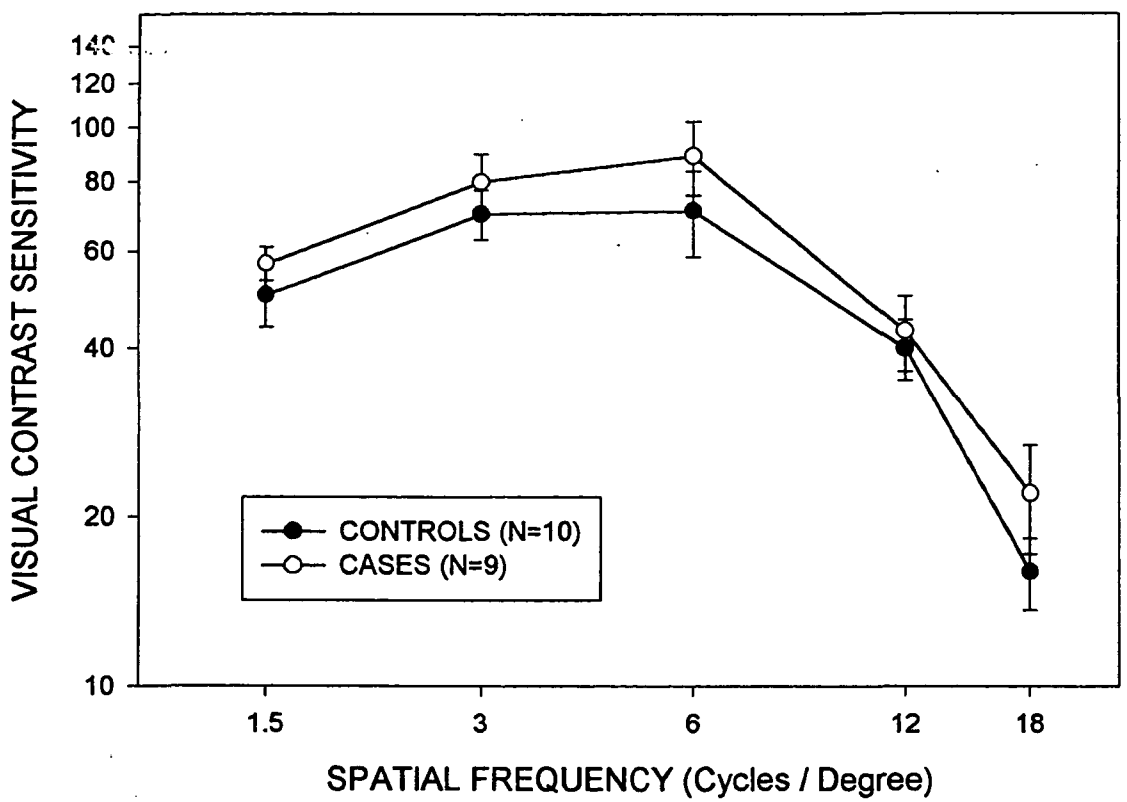
# VISUAL CONTRAST SENSITIVITY: FULL GROUP



# VISUAL CONTRAST SENSITIVITY: RESTRICTED GROUP



# VISUAL CONTRAST SENSITIVITY: HOT LINE



# A Biologically-Based Dose-Response Model

***Research to Improve Human-Health Risk Assessments***