Effect of Neurotoxicity in the Visual Function of Dry Cleaners

Efecto de la neurotoxicidad en la función visual de trabajadores de lavado en seco

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INTRODUCTION

Dry cleaning workers are exposed to organic solvents, particularly perchloroethylene (PERC), which is one of the most important ones used in this kind of industry. It affects the Central Nervous System (CNS), causing neurotoxicity (Spencer, 1985; World Health Organization, 1985; Candura, 1991). Neurotoxicity is defined as "the ability to induce adverse effects on the CNS and peripheral nerves or sense organs" (Mergler et al., 1990). It manifests as a continuous group of symptoms and effects that depend on the nature of the chemical, dose, duration of exposure and individual characteristics, such as age and build, among others (Baker, 1994). Exposure to neurotoxic substances produces three major types of alterations: a) Sensory —a sense organ is affected; b) Motor— causing paralysis to a lesser or greater degree. c) Changes in learning ability, memory retention and mood (Baker, 1994). Although some substances have a special affinity for certain regions of the CNS, neurotoxins have widespread effects on cellular processes involved in membrane transport in intracellular chemical reactions, in the release of secretor substances (White, 1997).

Neurotoxic substances such as PERC can cross the blood brain barrier due to the higher lipid solubility and interfere directly with the neurological function. Because PERC is a lipid soluble toxin, its main effects are on the lipid structures of the nervous tissue, which have most abundance in the myelin covering of the axon and cell membranes of the neurons. The primary routes of human exposure to PERC are inhalation, ingestion and dermal contact (Hake & Stewart, 1977). PERC vapour is well absorbed following inhalation exposure but dermal exposure is markedly lower than for other solvents (Lomax, Ridgway et al., 2004).

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The visual system is affected by acute inhalation exposure to PERC (Cavalleri, Gobba et al., 1994). Visual neurological alterations related to the visual contrast sensitivity function (CSF) and colour vision loss have been associated with PERC exposure (Barone; Nakatsuka, Watanabe et al., 1992; Cavalleri, Gobba et al., 1994; Mergler, Huel et al., 1996; Muttray et al., 1997; Gobba, Righi et al., 1998; Gonzalez, Velten et al., 1998; Onofrj, Thomas et al., 1998; Sharanjeet-Kaur et al., 1998; Gobba, 2000; Schreiber, Hudnell et al., 2002; Boeckelmann & Pfister, 2003; Gobba, Righi et al., 2003; Ihrig, Nasterlack et al., 2003; Till, Rovet et al., 2003; Lomax, Ridgway et al., 2004; Benignus et al., 2009).

Contrast sensitivity (CS) describes the ability to distinguish differences in luminance. It can be reduced by a number of disorders and occurs even when conventional high visual contrast acuity measurement is unaffected (Hohberger, Laemmer et al., 2007). Thus, contrast sensitivity testing can reflect visual perception in everyday life more accurately than standard acuity tests (Ginsburg, 1981). Contrast Sensitivity evaluation has been used in the analysis of neural disorders caused by solvent intoxication. It has been considered as a sensitive indicator of any neural disorder or neurological damage (Regan, Silver et al., 1977; Beck, Ruchman et al., 1984).

The relationship between occupational levels of exposure to lipid soluble chemicals such as solvents and CS has been studied. Altman and Bottger (1990) and Mergler et al. (1991) have reported lower contrast sensitivity, especially in the intermediate frequencies in microelectronics workers exposed to organic solvents, compared to normal subjects. Ophthalmotoxic chemicals have been inferred as the most probable risk factor (Frenette, Mergler et al., 1991; Mergler, Huel et al., 1991). In another example, Donoghue et al. (1995) examined 16 patients diagnosed with toxic encephalopathy and found that contrast sensitivity was depressed. Painters exposed to organic solvents have also been found to have a reduction in CSF (Frenette, Mergler et al., 1991; Broadwell, Darcey et al., 1995; Campagna, Mergler et al., 1995; Donoghue, Dryson et al., 1995; Echeverria, White et al., 1995; Castillo, Baldwin et al., 2001; Böckelmann, Lindner et al., 2003; Boeckelmann & Pfister, 2003).

Non-occupational levels of exposure to solvents have also been studied. However, only one study found that apartment residents and day care workers in New York City who were located near a dry-cleaning establishment, hence indirectly exposed to PERC at perhaps higher than the general community level of exposure, had reduced contrast sensitivity compared to age and sex matched controls who had community levels of exposure to PERC. Indirect exposure to solvents is a risk factor for the development of neurological disease (Schreiber, Hudnell et al., 2002).

Contrast Sensitivity Function can be measured using different techniques. Common to all techniques is that contrast and spatial frequency of a grating is varied to determine threshold levels of contrast (Campbell & Robson, 1964; Campbell & Robson, 1968). The grating spatial frequency is manifest as a sine-wave grating and is defined by the sum of widths of one light and one dark bar of the grating. Spatial frequency depends on the viewing distance, and is expressed in terms of the number of cycles of the grating that occur over a particular distance (cycles per unit of visual angle; cycles per degree [cpd]) (Boeckelmann & Pfister, 2003). All the studies cited above regarding CSF and neurotoxicity assessed the CSF used chart-methods such as VCTS (Vision Contrast Test System), MCT (Multivision Contrast Tester) and FACT (The Functional Acuity Contrast Test), which are cost-efficient and quick to use. However, because these tests only use a limited range of contrasts and step sizes and result in one estimate of threshold for each spatial frequency, they are not as precise as a computerized CS test, which employs staircases with smaller step sizes and uses at least (4) reversals to estimate threshold (Wacksman, 2007). Therefore the aim of this study was to determine the deficits in CS by spatial frequency in a group of dry cleaners who are exposed to occupational levels of PERC and its relationship with neurotoxic symptoms using computerized psychophysical testing techniques.

MATERIALS AND METHODS

Design of the study

A case-control study of visual contrast sensitivity and neurotoxicity in people occupationally exposed to PERC (dry cleaners) and people with community exposure to PERC was conducted in Bogota, Colombia. Informed consent was obtained from all subjects after the nature of the procedures was fully described. All studies were done with approval from the Human Research Ethics Committee University of New South Wales, Sydney, Australia and the Research Ethics Committee from the Health and Sciences Faculty of Universidad de La Salle Bogota-Colombia.

All visual function was examined monocularly (using the eye with the worst visual acuity) under standardized conditions and by the same examiner. Participants were refracted using auto refractor equipment (Topcon KR-3000); subjects who needed refractive correction were corrected with untinted lenses while doing the computerized CSF test. A Log Mar chart was used to assess distance visual acuity and a reading chart was used to evaluate near visual acuity (40 cms). Biomicroscopy (Care optical SLM-J/1/2/2E/2L) was used to evaluate the presence or absence of lens opacities, using the lens opacities classification system III (LOCS III) (Chylack, Wolfe et al., 1993). Direct ophthalmoscopy was assessed in order to establish eye normality (retina and ocular surface). Data regarding the typical number of alcoholic drinks and cigarettes consumed per day was collected because CSF is associated with reductions for all spatial frequencies in people who drink and smoke (Roquelaure, Le Gargasson et al., 1995; Pearson & Timney, 1998). Moreover, alcohol's metabolism in the body is similar to PERC (ATSDR, 1993; Newcombe, 2000; U S Environmental Protection Agency, 2008) and alcohol consumption is also known to reduce C (Nicholson, Andre et al., 1995; Roquelaure, Le Gargasson et al., 1995; Andre, 1996; Ferreira & Timney, 2004). These are consequently confounding factors for between group differences of CSF.

Participants were recruited in Bogota, Colombia. The inclusion criteria for cases in the study were: 18-40 year old adults who worked at dry cleaning establishments for at least one year. The inclusion criteria for controls were: 18-40 year old adults who have never worked at dry cleaning establishments and never use any organic solvents in their work. The exclusion criteria for both the cases and controls were: congenital colour vision deficiencies, systemic and neurological diseases unrelated to environmental toxics, macular diseases, and cornea or lens opacities. There were additional exclusion criteria for the controls including: normal visual acuity (better than 0.1 log MAR), have never worked in the dry cleaning industry, never lived with anyone who either works in the dry cleaning establishment or worked with any organic solvents, not living in the same building as a dry cleaning establishment. As controls resident in the community may send their clothes to be washed and ironed at dry cleaning establishments, this is another vector by which people can be exposed to PERC, so data regarding the frequency of use of dry-cleaning establishments for laundry was also collected as a possible confounding factor.

The number of participants was estimated based on a paper from Colombia entitled in Spanish: "Exposición ocupacional a solventes orgánicos y alteraciones en la visión en trabajadores de una empresa de hidrocarburos". Sample size estimated was thirty-five cases (35 dry cleaning workers) and 35 controls. It was calculated based on a power of 80%, confidence level of 95%, and odds ratio of 4.26. Final participant numbers were 40 dry cleaners and 35 controls.

Visual Contrast Sensitivity Stimuli

The CSF was assessed using a two alternative forced choice procedure using an oriented Gabor patch, as shown in Figure 1. The task of the observers was to indicate the orientation of the Gabor pattern (which was either tilted to the left or to the right at 45 degrees) presented on a grey background (55 cd/m^2). The contrast of the stimulus, coinciding with the amplitude of the gabor stimulus, was modified using a staircase procedure that corresponded to the 79% correct performance level. Initially the starting contrast of the stimulus was 0.8 and the step size was 0.08. After the first and subsequent reversals the step size was halved. After the third reversal the step size was 0.01 and remained at this value until the end of the staircase trial. The staircase lasted for 6 reversals and the average of the last 4 reversals provided an indication of the contrast detection threshold. No feedback was given to indicate the correctness of response. Viewing distance was 70 cm and all tests were conducted monocularly. The CSF test was generated on an Apple Macintosh Mac Book Pro computer and was programmed using MATLAB (The math works, Inc) with the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997). The abovementioned staircase procedure was repeated for the following spatial frequencies: 0.5; 1.0; 2.0; 4.0; 8.0 and 16.0 cpd.

Neurotoxicity Symptoms

Neurotoxicity was assessed using the neurotoxic symptoms questionnaire Q16 (Lundberg, Högberg et al., 1997) version modified to Likert scale (Jimenez, Khuu Sieu et al., 2011). The Q16 questionnaire is commonly used to monitor early effects of neurotoxic exposures in working populations (Lundberg, 1997). It contains 16 short questions on symptoms commonly described by workers exposed to solvents such as: "I have a short memory"; "I often have a painful tingling in some part of my body"; "I feel that I have less sensitivity or a complete loss of sensitivity in some parts of my arms or legs", graded for agreement using a Likert scale. Psychophysical CSF test and the modified Q16 questionnaire were tested for repeatability in preliminary experiments (Jimenez, Khuu Sieu et al., 2011).

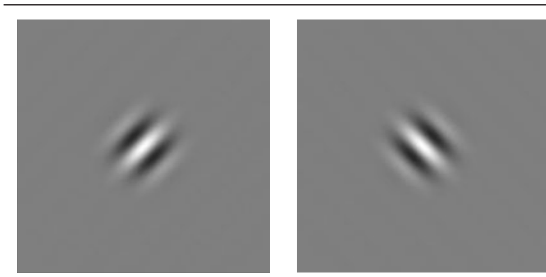

FIGURE 1. **Two orientations forced choice gabor stimulus of the computerised contrast sensitivity test**

Statistical Analysis

A repeated measures ANOVA was applied in order to analyse the differences of CSF between groups. A significant interaction between group and spatial frequency was found, and therefore the two groups were treated separately and the independent "t" test was applied in order to test for between-group differences in CS for each spatial frequency. Pearson correlation was used to analyse possible correlations between alcohol consumption and smoking with CSF by spatial frequency and group. Linear regression modelling was also used to determine the relationship between alcohol, smoking and neurotoxic symptoms on spatial frequency. The Enter (regression) and stepwise selection strategies of independent variable selection for the creation of linear regression models will be used for the spatial frequency with the largest mean difference between the control and cases. Standardized β will indicate the extent to which the variable or combination of variables are predictors of CS at 2 cpd spatial frequency. Non-parametric tests of significance were used (Mann-Whitney U test and Spearman correlation) to analyse Q16 modified version data. All analyses were conducted with the SPSS statistical package.

RESULTS

Characterization of the Group Sample

The mean age of the subjects in the occupationally exposed group (dry cleaners) was 33.2 years (SD±6.6). The mean period of exposure of cases to organic solvents (PERC) was 7.5 (SD=7.1) years. The percentage of cases who smoked was 12.5%, alcohol consumption was 25% and medication 20% for allergies and headache. In the control community exposure to PERC group, the mean age was 32.8 years (SD±7.21) and the percentage of cases who smoked was 23%, alcohol consumption was 46% and medication 8.6% for allergies and infections. More females were present in both groups (30 dry cleaners; 22 controls). There was no correlation between smoking by group and spatial frequencies evaluated (p>0.05).

Visual Contrast Sensitivity

Mean contrast sensitivity scores at all spatial frequencies and standard deviations are presented in Figure 2 for both groups. A repeated measures ANOVA with Greenhouse-Greisser correction determined that mean CSF differed statistically significantly between spatial frequencies (F (3.51, 256) = 93.53, P<0.0005). There was a significant effect on CSF by group (case or control), Wilks's Lamba= 0.73, F (5, 69) =4.98, p=0.001 and a significant interaction between group and CSF. Therefore, between-group differences in CS were assessed for each spatial frequency using the independent t-test and applying the Sidak-Bonferroni correction for multiple comparisons. Significant between-group differences (p<0.05) were observed at 1.0 cpd (167.45 ± 75.88 vs. 120.69 ± 90.30), 2.0 cpd (183.98 ± 63.49 vs. 97.53 ± 92.04), 4.0 cpd (161.12 ± 84.55 vs. 90.25 ± 102.67), 8.0 cpd (84.89 ± 68.25 vs. 30.75 ± 52.79), 16.0 cpd (15.01 ± 18.26 vs. 5.08 ± 2.45) but not at 0.5 cpd (127.67 ± 69.21 vs. 123.87 ± 88.70).

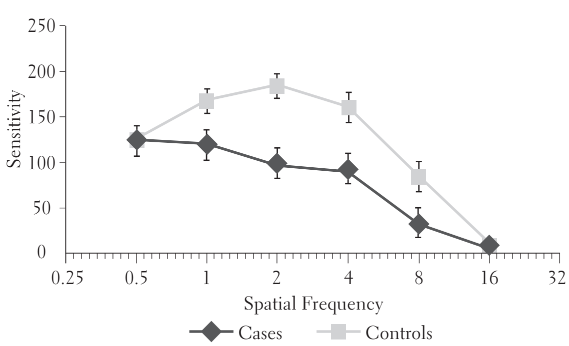

FIGURE 2. **Contrast sensitivity results for dry cleaners and controls. Vertical bars correspond to standard deviation**

Modified Q16 Neurotoxic Symptoms Questionnaire

There were significant correlations between the Q16 questionnaire modified version score and CS at spatial frequencies 2.0 cpd (RHO= - 0.27, p=0.017); 4.0 cpd (RHO=-0.23, p=0.04) and 8.0 cpd (RHO=-0.25, p=0.03) but not for 0.5 cpd (rho= 0.01, p=0.90); 1.0 cpd (RHO= -0.21, p=0.06) and 16.0 cpd (RHO=-0.16, p=0.15) for the case group. The mean score +/- SD for dry cleaners was 19.35 ± 13.02 and 13.8 ± 14.43 for controls. As expected, the dry cleaners had a significantly higher score (Mann Whitney U, p<0.05).

Effects of Frequency of Alcohol Consumption and Use of Dry Cleaning Services on Case and Control CS at 2 cpd Spatial Frequency

There was a significant negative correlation between alcohol consumption and spatial frequency 2.0 cpd (r=- 0.40, n=35, p=0.015) in the control group and the results (See Fig. 3) show a depression in CS at 2 cpd compared to age-matched normal values from other studies (Arundale K 1978; Sekuler & Owsley, 1983) suggesting that our control group may include people whose alcohol consumption rate has had an effect on their CS. Contrast sensitivity scores required transformation (square root) to achieve normality and the subsequent linear regression modelling confirmed that in the control group, alcohol is a significant predictor of decreased CS at 2 cpd. A significant model (F(1, 33)=6.24, p=0.02) for depressed CS at 2 cpd spatial frequency was -0.404x (daily alcohol consumption). The numbers of cigarettes smoked daily, age and frequency of use of dry-cleaning establishments for laundry were excluded from the model. For the cases, two significant models were generated using the linear regression modelling. The best fitting model (F(2, 39)=5.671, p=0.007) also confirmed that alcohol consumption was a predictor of poor CS at 2 cpd together with their use of ironing services from dry cleaning establishments. The model generated was -0.328X (daily alcohol consumption) + -0.366x (frequency garments sent to be ironed at dry cleaning establishments).

When the cases and controls were considered altogether as one group, linear regression resulted in a significant model (F(2, 74)=17.736, p<0.001), where group category (case or control) was the stronger predictor (standardized beta=-0.437) of depressed CS than daily alcohol consumption (-0.290). This indicates that alcohol consumption results in even worse visual deficits in dry cleaners, suggesting an additive effect in the toxic effects of PERC and alcohol.

DISCUSSION

In this research, the mean values of the CSF in the exposed group (dry cleaners) were significantly lower than in the control group above 1.0 cpd. This finding suggested that the organic solvent PERC could induce damage to the CSF and that it could be selective. Altman and Bottger (1990) reported lower contrast sensitivity, especially at 0.8; 1.0 and 2.0 cpd in microelectronics workers exposed to organic solvents, compared to normal subjects. Our results agree with these authors only for the spatial frequencies 1.0 and 2.0. Altman and Bottger (1990) did not test down to 0.5 cpd and they assessed PERC and other neurotoxic chemicals, which may have different effects on the visual system compared to PERC. Mergler et al. (1991) detected that CSF was affected by chronic solvent exposure at 3, 6 and 12 cpd spatial frequencies. Although our study did not measure 3.0 and 12.0 cpd spatial frequencies, they fall within the same range of our measurements (Cambell & Green, 1965; Campbell, Johnstone et al., 1971). Other studies that measured CS in workers exposed to organic solvents or solvent mixtures, reported a reduction at all spatial frequencies >1.5 cpd (Frenette, Mergler et al., 1991; Mergler, Huel et al., 1991; Broadwell, Darcey et al., 1995; Campagna, Mergler et al., 1995; Donoghue, Dryson et al., 1995; Echeverria, White et al. 1995; Castillo, Baldwin et al. 2001; Boeckelmann & Pfister, 2003), which is in agreement with our study. These studies, however, did not assess spatial frequencies below 1.50 cpd so it is not known whether our lack of a significant difference at 0.5 cpd is only specific to PERC.

In our study, the cases and controls did not present significant differences at 0.5 cpd, and both perform better at 0.5 cpd than in the Sekular study (1983). The possible reason for this phenomenon could be related to the kind of stimulus that was used in this study, short duration rather than long duration stimuli used by Sekular (1983). Short duration, low spatial frequency stimuli would preferentially stimulate the magnocellular system, which may explain the difference in the 0.5 cpd results (Graham, 1989) comparing with stationary grating that was used in the Sekuler & Owsley (1983) study. It is generally known that contrast encoding within the visual system is mediated by alternative processing streams, the magnocellular and parvocellular pathways with different response properties (Kaplan, Lee et al., 1990; Merigan & Maunsell, 1993; Lee, 1996). At the level of the retina and lateral geniculate nucleus, the magnocellular pathway has high contrast gain and approaches saturation at relatively low levels of contrast; additionally it is more sensitive in the detection and discrimination of briefly presented achromatic patterns of low contrast and low spatial frequencies. While the parvocellular pathway has a more linear contrast response function that extends to high contrast levels moreover, it is thought to mediate visual resolution and chromatic processing (Lennie, 1993; McAnany & Alexander, 2006). Considering the above, our results are in agreement with previous studies which resulting in reduction of CSF at intermediate and upper spatial frequencies that indicated that both magno and parvocellular pathways could be affected but that the parvocelluar may be more greatly affected than the magnocellular pathway.

Our case and control data indicated that alcohol consumption can reduce CS and in the cases, it is an additive effect according to linear regression modelling. In the controls, the depression in CS at 2 cpd is marked compared to age-matched normal values from other studies (Arundale K 1978; Sekuler & Owsley, 1983) and agrees with other studies of examining alcohol consumption and CS (Nicholson, Andre et al., 1995; Roquelaure, Le Gargasson et al., 1995; Ferreira & Timney, 2004).

Is the reduction in CSF of dry cleaners a functional concern? Comparing our results with population age-normal values for the CSF, the cases show a significant reduction at more spatial frequencies of a level similar to people between the ages of 70 and 80 (Sekuler & Owsley, 1983) despite being much younger (33.2 +/- 6.6 years). Figure 3 is a plot of our findings superimposed on replotted age normative data from Sekuler & Owsley (1983). The deficit in CSF displayed by cases from occupational exposure to PERC is approximately half that of age-matched people exposed to only community levels of PERC. According to Arundale K (1978), people with normal vision who are over 45 years old are less sensitive at mid and high frequencies than people with normal vision aged 18 – 39 years old and essentially equally sensitive to 0.5 cpd. This behaviour is similar to the findings related to dry cleaners in our study; however, the average age of dry cleaners was 33.2 years (SD±6.6), also indicating a visual system displaying functions similar to an older age group.

This CSF loss can affect different activities of daily life in dry cleaners (cases). People with reduced decreased CSF are known to have a high difficulty with high-risk driving situations than patients who have normal vision (MCGwin, Chapman et al., 2000), with tasks requiring distance judgment, night driving and mobility (Rubin, Roche et al., 1994). Other everyday tasks can be affected by vision contrast sensitivity loss, such as face recognition (Owsley, McGwin et al., 2001). Reductions in CSF are also associated with increased risk of falling, perhaps due to greater difficulty detecting low contrast objects and through effects on posture, balance and gait (Wood, Lancherez et al., 2009; Wood, Lancherez et al., 2011). Figure 4(a) shows the way that a person with normal contrast sensitivity may perceive a scene. Figure 4(b) represents a simulation of how the average dry cleaner sees the same scene

The Q16 modified version score showed a significant correlation with spatial frequencies 2.0, 4.0 and 8.0 cpd, being consistent with the notion that PERC exposures is associated with deficits in CSF at the middle and higher frequencies. This result has not been reported in the past. The Q16 modified version score was higher in dry cleaners (19.35) than controls (13.8) showing a significant difference (p<0.05). It suggests that occupational

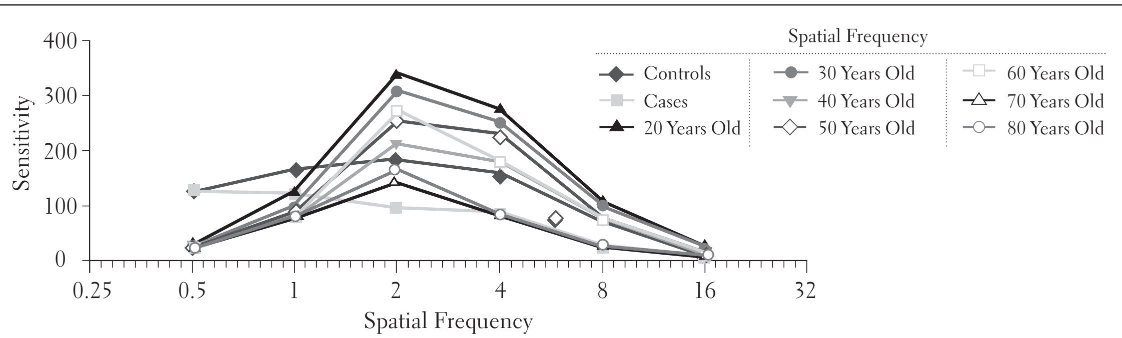

FIGURE 3. **CSF by age comparing with controls and cases Bogota, Colombia 2011. Age normative data**

FIGURE 4. (a) Scene as viewed by a person with normal CSF; (b) Scene as viewed by dry cleaners (simulation) with reduced CSF

levels of exposure to PERC are positively associated with neurotoxic symptoms including visual symptoms.

CONCLUSIONS

In conclusion, our results indicate that dry cleaners had poorer CSF than controls at spatial frequencies above 1.0 cpd and agrees with other studies (Altmann, Bottger et al., 1990; Mergler, Huel et al., 1991; Frenette, Mergler et al., 1991; Broadwell, Darcey et al., 1995; Campagna, Mergler et al., 1995; Donoghue, Dryson et al., 1995; Echeverria, White et al., 1995; Castillo, Baldwin et al., 2001; Boeckelmann & Pfister, 2003), and has extended the findings to 0.5 cpd. Alcohol consumption was found to decrease CS depression in both groups and this was an additive effect in the cases. Additionally, dry cleaners perceive the contrast at approximately the same level as people aged 40 to 50 years older that is aged 70 to 80 years old who are not drycleaners. The presence of neurotoxic symptoms in dry cleaning workers is associated with losses in CSF at middle and upper spatial frequencies, indicating that the reduction in CSF observed is associated with neurotoxicity. This loss of CSF has implications in daily life, for example driving; walking and capacity for face recognition. The CSF combined with the Q16 modified version are useful indicators of neurological function. The CSF appears to be a sign of neurotoxicity.

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