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Summary Report of the Peer Review Workshop on the Neurotoxicity of Tetrachloroethylene (Perchloroethylene) Discussion Paper

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
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NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency (EPA) policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This report was prepared by Versar, Inc., an EPA contractor (Contract No. 68-C99-238, Task Order No. 66) as a general record of discussions during the Peer Review Workshop on the Neurotoxicity of Tetrachloroethylene (Perchloroethylene) Discussion Paper. As requested by EPA, this report captures the main points and highlights of discussions held during plenary sessions. The report is not a complete record of all details discussed nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear. Statements represent the individual views of each workshop participant, none of the statements represent analyses by or positions of the EPA.

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INTRODUCTION

The Peer Consultation of *Neurotoxicity of Tetrachloroethylene (Perchloroethylene) Discussion Paper* was held on February 25, 2004, at the Marriott Crystal City Hotel in Arlington, VA. This one-day meeting was organized by Versar, Inc., for the U.S. Environmental Protection Agency's (EPA's) National Center for Environmental Assessment (NCEA). The purpose of the meeting was to provide expert commentary on the document and other issues relating to neurotoxic effects of tetrachloroethylene, or perchloroethylene (referred to in this report as "perc").

The agenda for the peer consultation (Appendix A) was developed by Versar and the Chair to allow for extended discussion sessions according to the charge questions. Specifically, the meeting began with welcomes from Versar and NCEA's Director of the Washington Division, introductions of the expert panel members, and overviews of the major comments on EPA's report. The majority of the day was devoted to the panel members' responding to the charge questions. Toward the end of the day, an observer comment period was held to allow the public to provide input on the document (the observers are listed in Appendix B). The meeting concluded with the Chair highlighting the expert panel's major comments and recommendations.

A five-person expert panel was assembled by Versar for the peer consultation, having experience in: epidemiology (studies of human neurological effects, specifically studies of visual function including visual contrast sensitivity), neurotoxicology and/or neurobehavioral evaluation (testing of human subjects for chemically induced deficits in nervous system performance, especially with solvents such as perc), and studies of the relationship between neurobehavior and low-level chemical exposures in residential or occupational populations. The members of the expert panel were

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This report summarizes the expert panel's recommendations and suggestions pertaining to EPA's document, *Neurotoxicity of Tetrachloroethylene (Perchloroethylene) Discussion Paper*. It includes

- Chair's Summary - presents a summary of the discussion from the meeting and other input from the expert panel on EPA's document.

- Individual Reviewer Comments - Each expert panel member provided written premeeting comments evaluating EPA's document and responding to the charge questions. Several of the panel members revised and expanded their comments following the meeting.

- Appendices - present the agenda and list of observers from the meeting.

Chair’s Summary

Summary Report of the Peer Consultation of
Neurotoxicity of Tetrachloroethylene (Perchloroethylene)
Discussion Paper

General Comments:
The EPA review document is clear, straightforward and addresses the issues in simple language without talking down to the audience. It is very well written. However, for the expert panel and for the future audience of EPA Risk Assessors, the document can and should be improved. The document is uneven in its presentation. For example, visual tests have a more extensive treatment than do the other measures and some reviewers felt the implications were overstated (e.g., impairment in children by contrast sensitivity loss; discussion of spatial frequency and small and large objects is not consistent with current theory). One reviewer disagreed, as noted below, because visual effects may be the critical or earliest effects of Tetrachloroethylene or Perchloroethylene (Perc) and thus it is appropriate for them to have the most extensive treatment. Thus, for this reviewer, the implications may be appropriately strong. Table 1 is a valuable tool for grasping the details of the array of studies described in the document. However, numerous additional details are needed to allow the reader to compare the studies. In the rows below the current Table and in the section of the document summarizing each study, bullet-level notes should be added to describe or address study design; the groups and the recruiting strategy; potential biases including motivational issues; statistical tests and power, summary of strengths and serious weaknesses, and conclusions. These additions will allow readers to see all the key information in a single view.

Charge a. What are the relative strengths and limitations of the existing human studies of the neurological effects of Perc (e.g. sample size, statistical power, potential biases, biological or clinical relevance of the findings, degree of consistency)? Do the EPA materials adequately evaluate these issues?

Expert Panel Responses:
Of the specific issues posed in the charge, statistical power is not mentioned in the review of the individual studies, although it is alluded to in the summary statements. Biological or clinical relevance is detailed for visual contrast sensitivity but not for most other measures, which makes for an uneven presentation. Potential biases are identified in some cases, but they are not mentioned in other cases, leading one to question if they were assessed. Motivation is a concern in any epidemiological study, but unaddressed in the document. The statistical analyses of the various studies present a huge diversity from modern, sophisticated and appropriate analyses (regression) to crude analyses that are susceptible to multiple comparison errors. (This general point was echoed by one of the meeting observers.) The document would be substantially improved by listing these points at the end of the description of each individual study, as noted above.

The summary on page 26 begins with the key point: It is important to compare performance across studies to identify patterns of deficits. The expert panel agreed that

this was the appropriate approach to evaluating such data. An alternative approach of eliminating all flawed studies and then drawing conclusions from the remaining few studies was not favored because epidemiologic research is often flawed, yet value and richness can be found when consistency emerges from the diversity and despite the flaws. One reviewer offered an informal rule of thumb that he considered findings compelling if there are consistent test results in 3 or more epidemiologic studies from different laboratories studying different populations exposed to the same compound, even if [different] serious weaknesses are identified in those studies. The table is very helpful in identifying such consistent patterns.

The animal studies should be related to the human studies and be included in the summaries. At present, they seem unconnected to the human research and their contribution to the review is unclear. Mechanism should be addressed where possible, and kinetics must be included to address threshold issues, addressed below.

The contrast sensitivity test employed an exemplary protocol and this could be one reason why it was sensitive to low level exposures.

Dr. Bowler expressed concern that the lack of sensitive tests of important functions affected by solvents is a limitation of all the human studies except the studies employing vision assessments and the Echeverria et al. 1995 study. Those more sensitive tests measure mood status, cognitive flexibility, information processing and attention and concentration, all of which are important early indicators of overexposure to organic solvents. Several of the studies used computerized tests, which, with the exception of reaction time, are not as sensitive and have not been shown to correlate well with existing standardized, normed, hand-administered tests. A caveat is that the psychometricians who administer "hand-administered" tests must be trained and overseen by well-trained and experienced neuropsychologists to assure the validity and consistency of the test results. Further, none of the studies included in the analyses the potential confounders of ethnicity (which affects neurobehavioral test scores) or measures of secondary gain potential. Children who are worried may reflect the parents' worries; better tools may be parent teacher rating scales (e.g. Achenbach). These omissions thus raise concern that thresholds identified in those studies may be above a true lowest effect level because they were not based on the most sensitive tests available.

In summary, the document does not fully evaluate a number of important issues, but it currently provides much of the critical information and has done so accurately.

Charge b. How consistent are the visual contrast sensitivity effects seen in one residential study (with two exposed groups) with findings of other visual effects seen in other occupational and residential studies (where visual contrast sensitivity was not tested)?

Expert Panel Responses:
The visual contrast sensitivity findings are not inconsistent with the findings of other

visual system effects, but most panel members felt that the document goes too far to conclude that they suggest a common mode of action (p. 36). A second viewpoint was that there is substantial though still inconclusive evidence that the eye is the critical organ affected by Perc, so that the focus of the document should be on the visual system. These viewpoints diverge somewhat on the appropriate emphasis of the document but agree that the evidence is not yet conclusive.

In general, the occupational studies share a more common set of tests in their test batteries. This consistency (Lauwerys et al, 1983; Seeber et al., 1989; Echeverria et al., 1995; Ferroni et al., 1992) is to be expected because selected tests are from common domains recommended by the World Health Organization, the European Union, and ATSDR. The residential studies use more diverse measures and, at least within the set of studies included in this review, are more oriented to using physiological tests such as the Lanthony Color Hue Test or Color Perception Test, or Contrast Sensitivity.

With respect to adverse effects on visuospatial function (pattern memory, pattern recognition, Benton, and visual reproductions), there are reports in high- and low-exposed populations of effects in visually mediated pathways (Seeber et al., 1989; Altman et al., 1995, and Echeverria et al., 1995). The results across these three studies appear reasonably consistent despite substantial differences in study design. Further, Echeverria and colleagues more comprehensively confirmed these findings in an independent sample of dry-cleaners in a follow-up study (see Echeverria et al., 1994).

A second independent line of evidence can be found in both the occupational and residential studies (Nakatsuka et al., 1992; Cai et al., 1991; Cavalleri et al., 1994), all of which evaluated performance on the Lanthony color vision test. Evidence of a trend in the expected direction was established for a high exposed group but not a low exposed group (Cavalleri et al., 1994) among dry cleaners. Adverse effects were also found in a residential population though it was not statistically significant (Schreiber et al., 2002). The exception was the one study by Nakatsuka et al. (1992) that did not find an effect. This study used the Lanthony new color test as a screening tool that was followed by a more diagnostic assessment among those with positive findings using a clinical color vision test. This tiered approach eliminated false positives. However, there were significant weaknesses in this study (variable lighting conditions, varying training of test administrators, lack of matching for age and alcohol consumption) that reduce the weight that may be given to the study.

Lastly, visual contrast sensitivity deficits in Perc-related spatial function were observed at very low exposure concentrations in residential populations that are subject to very different dose-rates (Schreiber et al., 2002) than that incurred by occupational workers. This contrast sensitivity study showed an impressive and consistent loss, and contrast sensitivity may have been more sensitive than other measures. This could reflect a particular sensitivity of the visual system to Perc. However, it may be more likely that this test (psychophysical procedure) was simply carried out by a superior test method than was the case in other tests in other studies. Specifically, Schreiber et al.'s contrast

sensitivity test used many trials of forced-choice testing ("was the grating tilted to the right or left?") rather than asking if the grating was seen. The forced-choice method is the most reliable and consistent way to measure visual performance. It is possible that the contrast sensitivity loss may reflect impaired function throughout the brain, since contrast sensitivity is affected by retinal, optic nerve, or central brain dysfunction. The color confusion findings in other studies could also reflect a generalized disorder of nervous system function, since color vision can also be decreased by effects at many locations in the visual system. It is possible that color confusion, if it had been tested with as robust a psychophysical test procedure as was used to test contrast sensitivity, might have been equally sensitive to Perc. Nonetheless, this unique observation is potentially important and should be replicated, particularly at very low PCE exposures (in the ppb range) reported in the Schreiber et al. study.

Overall, the evidence reveals a high degree of consistency in visually mediated function but does not conclusively demonstrate a common mode of action. The panel supports an emphasis on the visual system but some felt the current treatment went too far in that it left a somewhat subjective impression that the visual system effects provided the only critical findings.

Charge c. Table 1 of the EPA document provides a summary of types of neurological tests that have been conducted measuring different effects with different populations exposed to Perc. What is the biological and or clinical significance of the measured endpoints in these different studies?

Expert Panel Responses:
The tests reflecting deficits relate to functions required for basic species survival (e.g., signal detection against a background of noise, confusion of colors, memory for visual stimuli) and thus have high biologic significance. Further, the tests in the human studies that measure a range of sensory and cognitive functions are widely used, are reliable and have demonstrated sensitivity to neurotoxic agents, including solvents, in cross-sectional studies (e.g., Anger, 2003). The tests have been used in clinical and experimental research to assess normal and abnormal nervous system functioning. However, the deficits are nonspecific in that no specific functional deficits can be identified. There is an indication of greater specificity from a review of papers some of which are not included in the EPA document (see comments by Echeverria and Merigan in the responses to charge h).

Charge d. What weight should be attached to reported findings of neurological effects in residential populations at exposure levels below those seen in the occupational studies?

Expert Panel Responses:
The panel saw strengths and weaknesses in the residential studies. The visual contrast sensitivity results in the Schreiber et al. study appear to be robust and the result of tests administered appropriately.

Both residential studies, however, have serious problems in their control groups. The recruitment methods and any potential interest in secondary gain (motivation to perform poorly) are not addressed well in either study. More importantly, the educational differences in the Altmann et al. study are not described but must be large from the distributions noted in the article. Educational differences were not addressed in the Schreiber et al. study. The Schreiber et al. controls were drawn from acquaintances (day care staff study only) and employees of the NY state Department of Occupational Health and US EPA, who could have had considerably more education than the exposed populations and different occupational backgrounds (perhaps even controls who were employed and exposed who were not employed). The relatively crude adjustments for education in the Altmann et al. study might not have been sufficient to adjust statistically for the true education differences, and this is a major issue for the cognitive tests used by Altmann. Education would have less impact on the tests (visual contrast sensitivity) in the Schreiber study, but the differences in occupation could have had more impact. Some of these issues could be cleared up by contact with the investigators. Drs. Schreiber and Hudnell were both in the audience at the meeting and questions were directed to them about this study. Their oral comments provided suitable answers to some of the questions about the Schreiber et al. study, but written answers are needed for careful review by EPA. We recommend that EPA pose a series of questions to Dr. Schreiber to clarify recruitment, composition and motivation of the control groups in her study.

While the sample size is small in the Schreiber et al. study, the robustness of the findings at the very low levels of exposure relative to the occupational studies, were jarring and concerning. Three factors set the residential studies apart from the occupational studies: (a) the very extended time course of the exposures in a residential setting (up to 24 hours per day and up to 7 days per week vs. 8 hours per day at 5 or 6 days a week in most occupational settings/studies); (b) the slow elimination of Perc (e.g., excretion of the unchanged compound at 3 different rate constants, with half-lives of 12-16, 30-40 and approximately 55 hours, per Monster et al., 1979) combined with its lipophilic properties and low volatility that will further increase body burden (per Filser and Bolt, 1979); and (c) the potential for susceptible populations to be present. These factors add greater weight to the concern and dictate extraordinary consideration of this one study.

The lead author of the key study, Dr. Schreiber, a member of the audience, expressed her opinion that the evidence provided by the residential studies, did demonstrate that there are health effects at the low concentrations reported in the residential studies. The expert panel did not appear to agree on this point. Some were unwilling to accept this viewpoint without a replication (a second study finding the same effect), some appeared to reserve judgment until additional data on the controls were thoroughly reviewed and others were closer to Dr. Schreiber's judgment. However, clearly all panel members were concerned that the uncertainty presented by the many unknowns could lead to highly damaging exposures to sensitive populations. Further, the adverse effects would not be detected by parents or schools who would have no comparison basis and it is unlikely that a population-based study would be conducted in such populations to reveal that damage.

Charge e. Do the epidemiology studies identify susceptible populations, and in particular do the residential data indicate that children and elderly people may be more susceptible to the effects of Perc?

Expert Panel Responses:
As in the response to the previous charge, added data from Dr. Schreiber could support a more definitive answer to this question. Panelists identified additional research studies that were not reviewed in the EPA Perc document that related to this question. These data suggest an increased susceptibility to PERC neurotoxicity during the latter part of pregnancy and during early life (Beliles, 2002). An effect on both neurobehavioral tests (Till et al, Neurotoxicology and Teratology, 2001) and visual function (Till et al., teratology, 2001) resulted following occupational exposure of mothers to various solvents, including PERC, during pregnancy. Abnormal chromatic responses and reduced contrast sensitivity has been observed in a 2.5-year-old boy following prenatal exposure of the mother in dry cleaning shop to Perc levels reportedly within regulated limits (Till et al. 2003).

Charge f. Do the studies reporting decrements in neurological function (including vision) in people exposed to organic solvents add support to conclusions about the hazards of Perc?

Expert Panel Responses:
The Perc studies need to stand on their own, an issue discussed next. However, the broad and deep solvent literature does identify a range of adverse health effects following chronic solvent exposure that include those reported following Perc exposures. A lack of common findings would diminish confidence in the Perc research because the Perc studies would not fit in an established context of demonstrated findings. Therefore, this similarity does add confidence in the Perc findings.

Evidence of adverse nervous system effects in populations exposed to solvents is extensive and compelling. Most relevant, Dr. Echeverria has reviewed the nervous system effects of the straight chain chlorinated solvents. There is evidence of persistent visual deficits following chlorinated solvent exposure (e.g., Broadwell et al., 1995). Complex visual process is a vulnerable target for CNS insult and has been evaluated with evoked potentials (e.g., Arezzo et al., 1985). Evoked potential changes have been seen following chronic chlorinated solvent exposure such as trichloroethylene (Fergusson et al., 1970). The observed disturbances in latency and amplitude of wave form components from a pattern stimuli of a checkerboard reversal are comparable to adverse patterns observed for clinical conditions such as multiple sclerosis and exposure to a number of chemicals, including methyl mercury, metallic mercury, nhexane, carbon monoxide, xylene, toluene and ethanol (e.g., Urban et al, 1999; Vrca et al., 1995; Altmann et al., 1998). Cognitive and related behavioral measures of nervous system deficit have been used extensively to study both acute (Dick, 1995) and chronic (Anger, 1990, 2003) adverse effects of a broad range of solvents. While the effects are equally broad in the many studies reported to date (Dick, 1995; Anger, 1990, 2003), they do reveal adverse

effects on all the neurobehavioral tests associated with adverse effects following Perc exposure in the EPA document.

The consistency across the Perc and tricholoroethylene studies, at least with respect to visually mediated tests, warrants a future investigation of EEG and evoked potential activity to identify more specific neural pathways disrupted by Perc. This knowledge would advance future research into toxicologic mechanisms explaining a potential CNS syndrome due to Perc exposure in humans. However, at this time, the lack of a definitive mechanism makes it difficult to answer the question posed by this charge with great clarity.

Charge g. Can an association be made in the separate studies and in all studies collectively between Perc exposure and observed neurotoxicity? Does the set of studies as a whole indicate that Perc exposure to the general population presents a potential health hazard?

Expert Panel Responses:
The review panel asked for clarification of the charge. Based on the EPA staff responses, the panel broke the charge down into three questions, as follows:

Question g1: Do the studies reviewed in the document support the hypothesis that Perc is hazardous to the adult nervous system?

Expert Panel Answer: The pattern of results provides convincing evidence that Perc is hazardous to the adult nervous system.

Question g2: Can the findings in the occupational populations be generalized to the general US population, and specifically residential or community populations?

Expert Panel Answer: The general population is more heterogeneous than occupational populations. It includes susceptible sub-populations, including children, pregnant women and the elderly. Some members of the general population will receive a longer period of daily exposure, including virtually 24-hour per day exposure, 7 days per week or virtually continuous exposures. These issues suggest that the occupational exposures may mis-estimate and at worst substantially underestimate the effects of the exposures because the studies are conducted on shorter exposure durations (though of higher concentrations). Perhaps when duration becomes continuous and clearance is slow, the effects of low exposure concentrations are greatly magnified. This is an issue for future research to address, as it represents a paradigm shift for occupational researchers when conceptualizing how exposure and duration interact. Thus, the panel does not have the basis for answering this question.

Question g3: Is there evidence of a dose response or an exposure effect gradient in the studies of Perc, and is there a threshold?

Expert Panel Answer: A dose-response relationship is supported by three findings: (a) in a study of dry-cleaning workers, a deficit was observed in dry-cleaners who were highly exposed to Perc, but not in ironers, who had lower exposures (Cavalleri et al., 1994); (b) a significant correlation ($r = 0.52$; $p<0.01$) observed between individual Perc exposure (environmental Perc levels measured using personal dosimeters) and colour vision impairment (quantitatively evaluated using the Color Confusion Index) (Cavalleri et al. 1994); and (c) the progression of the impairment observed in dry-cleaners whose exposure was increased (Gobba et al, 1998).

Dr. Echeverria's conclusion, which is informed by extensive experience studying the neurobehavioral effects of a number of solvents and other compounds, is that there is a threshold (where some agents including styrene do not appear to have thresholds; e.g., Campagna et al., 1996). That threshold lies in the range of 5-20 PPM in the occupational studies of Perc exposure in Dr. Echeverria's judgment. The residential studies, however, present a different exposure scenario and context than the occupational studies. Whether they may suggest a lower threshold was not discussed during the meeting, although comments sent subsequently by Dr. Gobba offered the opinion, based on data described in the response to charge d, that a lower exposure limit was justified for community populations using a traditional factor of 10 to account for susceptible individuals that can be found in such a population.

One panel member noted that the studies that formed the base for the review did not use the tests that are the most sensitive (i.e., occur at the lowest concentrations or appear before other effects) in solvent research. An example is mood for which there are reliable measures. Therefore, the threshold for the occupational populations may be lower than 5-20 PPM.

As might be expected from any group of research scientists presented with challenging questions about extrapolation and exposure thresholds, recommendations for further research that would clarify the issues were offered (although the EPA charges did not request them). Chief among the recommendations is to use more sensitive tests in residential (and occupational) studies of Perc exposure to identify both confounders and adverse health effects that would identify the lowest possible adverse effect levels. Specific recommendations include measures of mood status, cognitive flexibility, information processing and attention and concentration, all of which are important early indicators of overexposure to organic solvents. Replication of the Perc studies in residential populations was also suggested.

Charge: h. Are there any published studies or data relevant to the neurotoxic risk of Perc that are not included in the discussion paper?

Expert Panel Responses: The individual comments of Drs. Gobba, Bowler and Echeverria discuss several papers not mentioned in the EPA review. Aside from those mentioned above, none were felt to represent critical data omissions in the current EPA document, but rather added support and weight to the existing conclusions.

Some of Dr. Gobba's references are to papers published in English by well-regarded research scientists and should be reviewed and incorporated into the document, while other publications are in other languages. Dr. Gobba has provided summaries of these papers, and the panel recommends that EPA consider the summaries and have strongly relevant papers translated for addition to their review document.

Dr. Bowler has provided additional studies and a table that summarizes those findings and the findings already in the EPA review document, in her separate submission. This table may provide EPA with confirmation of their review and speed the task of expanding their own table, recommended above. Arlien-Soborg's 1992 CRC Press book on solvents is a classic recommended for general background reading by EPA, although it does not have a chapter specifically on Perc.

Dr. Echeverria provided a compilation of her research on Perc, including three published studies and three not yet published. EPA staff indicated they had a prior compilation that may encompass her findings. Since these studies also add weight and confirmation to the existing document, EPA should consider including all the published papers in their review (only one is in the EPA review now) and consider the unpublished papers to determine if critical data might answer key questions to merit the appropriately rare step of including unpublished data in their deliberations. One conclusion reached by Dr. Echeverria in that review is that tests that require vision are usually affected whereas the same test given in a format that does not require vision is typically not affected. In response to this point, Dr. Merigan noted that this would indicate a level of functional specificity not suggested above (charge c) The panel's response to charge c was that no specific functional deficits were demonstrated (e.g., vision tests require attention and detection of signals in noise, so any damage to any of several systems could be responsible for the changes). If supported by critical review, this specificity lends added support and thus added credibility to the conclusions in the EPA review document.

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Respectfully submitted,

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Individual Reviewer's Comments

Review by
Kent Anger, Ph.D.

Preliminary Comments by Kent Anger (February 19, 2004)

The ultimate purpose of this document is not clear, though it will lead to a "review of neurotoxicity" in a "comprehensive health assessment document (toxicological review document)" that will be released to the public. As such, it has much to appreciate but it also has an unevenness that is a concern. The strengths of individual studies are difficult to discern and the weaknesses are not consistently described. The document does not lead to clear conclusions, although it lays much of the base for those conclusions.

Charge a. **What are the relative strengths and limitations of the existing human studies of the neurological effects of perc (e.g. sample size, statistical power, potential biases, biological or clinical relevance of the findings, degree of consistency)? Do the EPA materials adequately evaluate these issues?**

Comment: The document is somewhat uneven in its discussion of key points. Of the specific issues posed in the charge, statistical power is not mentioned in the individual studies, though it is alluded to in summary statements. Biological or clinical relevance is detailed for visual contrast sensitivity but not for most other measures. Potential biases are identified in some cases, but they are not mentioned in other cases leading to a question as to whether they were assessed. The document would be improved by listing these points at the end of the description of each individual study (in the same order for every study), by issue. For example:
• Study design: Lack of control group; differences inferred from normative data that cannot be well matched to the exposed group.
• Potential biases: None noted.
• Statistical power: Had x power to detect effect in contrast sensitivity with the N of xx in the study.
• Serious weaknesses:
And possibly even—
• Conclusions: This study demonstrates an association between …, although the lack of description of the source of the controls reduces confidence in the finding.

The summary on page 26 begins with the key point: It is important to compare performance across studies to identify patterns of deficits. I would cast it in stronger terms. Where there are consistent findings in 3 or more epidemiologic studies from different laboratories studying different populations, the findings may be considered compelling even if [different] serious weaknesses are identified in those studies. The table is very helpful. The summaries, however, need to be focused by beginning with the most compelling findings and ending with a summary of what led to their conclusions. VCS is given too much prominence, which is to say other measures seem to be ignored and gives the impression that VCS provided the key finding. It is but one of many.

In sum, the document does not adequately evaluate the issues, but it has already provided much of the information and done so accurately.

Charge b. How consistent are the visual contrast sensitivity effects seen in one residential study (with two exposed groups) with findings of other visual effects seen in other occupational and residential studies (where visual contrast sensitivity was not tested)?

Comment: They are not inconsistent, but the document goes too far to identify these as suggesting a common mode of action (p. 36).

Charge c. Table 1 of the EPA document provides a summary of types of neurological tests that have been conducted measuring different effects with different populations exposed to perc. What is the biological and or clinical significance of the measured endpoints in these different studies?

Comment: The measures in the human studies are widely used and have demonstrated sensitivity to neurotoxic agents, including solvents, in human cross-sectional studies. The tests have been used in clinical or experimental research to assess normal nervous system functioning. They measure a range of sensory and cognitive functions.

Charge d. What weight should be attached to reported findings of neurological effects in residential populations at exposure levels below those seen in the occupational studies?

Comment: Both residential studies have serious problems in their control groups. The recruitment methods and any potential interest in secondary gain are not addressed well in either study. More importantly, the educational differences in the Altmann et al. study are not described but must be large from the groupings noted in the article. Educational differences were not addressed in the Schreiber et al. study. The Schreiber et al. controls were drawn from acquaintances (day care staff study only) and employees of the NY state Department of Occupational Health and US EPA , who could have had considerably more education than the exposed populations and different occupational backgrounds (perhaps even controls with and exposed without occupations). The relatively crude adjustments for education in the Altmann et al. study, might not have been able to adjust statistically for the true education differences, and this is a major issue for the cognitive tests used by Altmann. Education would have less impact on the tests (VCS) in the Schreiber study, but the differences in occupation could have had more impact. Some of these issues could be cleared up by contact with the investigators. Pending suitable answers, limited weight should be attached to the residential studies.

Charge e. Do the epidemiology studies identify susceptible populations, and in particular do the residential data indicate that children and elderly people may be more susceptible to the effects of perc?

Comment: The comment to d applies to e. Susceptible populations are always a concern and rarely studied. Here we have some interesting data but flawed study reports or perhaps flawed studies.

Charge f. Do the studies reporting decrements in neurological function (including vision) in people exposed to organic solvents add support to conclusions about the hazards of perc?

Comment: They add very limited support. Because the category of solvents includes such broadly different compounds is paired with so many different behavioral effects reported in the various studies, the relationship between those findings and the specific solvent perc is virtually impossible to assess. If a narrow range of solvents could be identified that are structurally similar to perc, the behavioral effects would be well worth examining.

Charge g. Can an association be made in the separate studies and in all studies collectively between perc exposure and observed neurotoxicity? Does the set of studies as a whole indicate that perc exposure to the general population presents a potential health hazard?

Comment: Yes. There is consistency in statistically significant results among the studies where common tests were used to label it as an association. A conclusion that perc is neurotoxic at low levels is justified. Any neurotoxic agent is a potential health hazard to a population exposed to it.

Charge: h. Are there any published studies or data relevant to the neurotoxic risk of perc which are not included in the discussion paper?

Comment: None of which I am aware.

Review by
Rosemarie Bowler, Ph.D., M.P.H.

Response by Rosemarie Bowler (February 23, 2004)

Charge a. What are the relative strengths and limitations of the existing human studies of the neurological effects of perc (e.g. sample size, statistical power, potential biases, biological or clinical relevance of the findings, degree of consistency)? Do the EPA materials adequately evaluate these issues?

Comment: In reviewing these studies I would like to comment that it is very well that only studies listing levels of Perc are used. However, most of these reports, with the exception of vision assessments and the Echeverria et al. 1995 study, lack sensitive tools to evaluate some of the other health effects of Perc. Most do not utilize any scales of symptoms or mood status. Mood status in organic solvent exposure is an important early indicator of overexposure, as is cognitive flexibility, information processing and attention and concentration. Despite listed effects in Table 1, p 39, it can be seen that few studies utilized these types of sensitive clinical neuropsychological screening tests. Several of the studies used computerized tests, which with the exception of reaction time, are not as sensitive and have not been shown to correlate well with existing standardized, normed, hand-administered tests. Other general comments: none of the studies mention ethnicity (which affects neurbehavioral test scores); those studies including neurobehavioral testing do not include any tests of motivation, there may have been secondary gain potential, tests of effort could control for this. Children who are worried may reflect the parents worries, better tools may be parent teacher rating scales (e.g. Achenbach).

Charge b. How consistent are the visual contrast sensitivity effects seen in one residential study (with two exposed groups) with findings of other visual effects seen in other occupational and residential studies (where visual contrast sensitivity was not tested)?

Comment: It appears that only one study (Schreiber et al. , 2002) reports positive contrast sensitivity findings in your Table 1, p 38. The additional article by Gobba et al., 1998 reports color vision changes as do the Schreiber (trend only) and Cavallieri studies study but the Nakatsuka et al., 1992, study does not report a postivie finding of color vision. I believe that these results reflect insufficient data and insufficient epidemiologic evidence but strongly suggest that color vision impairment, as would contrast sensitivity impairment, if they were tested in acutely exposed cases, chosen in an epidemiologically defendable manner, should be expected. In thousands of cases I have evaluated who have had organic solvent exposure, vision impairment in both color and contrast have been found.

Charge c. Table 1 of the EPA document provides a summary of types of neurological tests that have been conducted measuring different effects with different populations exposed to perc. What is the biological and or clinical significance of the measured endpoints in these different studies?

Comment: As can be noted from my own Table (coincidentally in a similar organizational scheme and sent to you prior), several more studies (listed in my table) have been published on reported neuropsychological findings. Similar to a) above, most studies lack a cohesive sensitive

and generalizable evaluation, utilized different types of tests, making a cross-comparison very difficult. Formal symptom and mood testing should be included in such studies. Symptom reporting appears to be more frequently present than does formal mood testing, utilizing sensitive tests such as the SCL90 R is essential. The BRFFS would be a useful adjunct with it's extensive national normative database. In terms of information processing, neither the Seeber nor the Altmann studies utilize sufficiently sensitive and established, standardized tests of information processing from neuropsychology, which are available for use. Digit span is reported only in total scores which loses its sensitivity. Digit span should be reported both for forward and backward digits separately and the difference between both should be examined. This loss of information is crucial, as one would expect digits backwards to be effected more than the forward spans.

Charge d. What weight should be attached to reported findings of neurological effects in residential populations at exposure levels below those seen in the occupational studies?

Comment: It is presumed that the two studies referenced are the Altman and Schreiber studies. As far as the Altman study is concerned, the selection method seems unbiased and sound and the results therefore raise some concern. However, only the NES battery was used, which is not as clinically sensitive as hand administered tests which may affect the level of findings as the subjects were only n=19. If computerized tests are used, I would pay more attention to such findings if they would represent larger samples. The Schreiber et al study represents findings in the occupational group which, however, had higher levels of exposure, but also reported findings in the residential group with lower levels of Perc. This suggests further research in environmental settings should be done with low level exposures, utilizing a sensitive neuropsychological battery but possibly a more stringent selection paradigm.

Charge e. Do the epidemiology studies identify susceptible populations, and in particular do the residential data indicate that children and elderly people may be more susceptible to the effects of perc?

Comment: Insufficient evidence according the research literature exists on special and susceptible populations. We do not know about pregnant women, the elderly and need to take into consideration existing studies of respiratory health effects.

Charge f. Do the studies reporting decrements in neurological function (including vision) in people exposed to organic solvents add support to conclusions about the hazards of perc?

Comment: Yes these studies do suggest that perc is a health hazard. Particularly the animal literature, supports the articles on human health effects. For humans it is necessary to ask about correction of eysight (glasses) as well as the age of their glasses and the date of the last prescription to ascertain how much of a correction in vision is possible.

Charge g. Can an association be made in the separate studies and in all studies collectively between perc exposure and observed neurotoxicity? Does the set of studies as a whole

indicate that perc exposure to the general population presents a potential health hazard?

Comment: Same as f)

Charge h. Are there any published studies or data relevant to the neurotoxic risk of perc which are not included in the discussion paper?

Comment: Other studies are cited in my Table which were provided. Additionally it was decided that a paper I published recently on ethylene dichloride (a chlorinated solvent) is relevant, which I therefore attach in pdf format.
Additionally, the committee may be interested While I had mentioned the Peter Arlien-Soborg Solvent Neurotoxicity book published in 1992 by CRC press, I note that there is not a separate chapter on Perc.

Table 1 (Part A)

No.	Literature Review of		Tetrachloroethylene (Perchloroethylene)								
			Population				Exposure	Methods	Tests used: Neuropsy		
			N	Ethnic	Control	Description	Acute/ Chronic	Type	Cog.	Memory	Motor, React. Time
	Authors	Year	#	Black White Other	Y/N		A=Acute C=Chron and duration	R=Review C=Clinical E=Epi F=Field	y/n, - indicates no info or no test given	(Attention & Concen.) y/n and list	y/n and rt=reaction time, ft=fingertap
1	Rowe et al.	1952	6	-	N	Six human volunteers were exposed to low doses of 106- 1060 ppm	A	C	-	-	-
2	Stewart et al.	1961	1	-	N	Enclosed work situation using a mixture of Stoddard's solvent and PER	A	Case study	-	-	-
3	Stewart et al.	1970	16	-	N	16 Volunteers were put into a inhalation chamber at an exposure of 100 ppm for 7 hours of one day(one group) and 7 hours for five consecutive days	A	C	-	-	-
4	Hake & Stewart	1977	1	-	N	Dry cleaner operator found lying in pool of PER	A	Case study	-	-	-
5	Hake & Stewart	1977	?	-	N	A: volunteer male exercises for 30 minutes and inhales 150 ppm on 2 separate days, then sedentary on day 3 B: several concentration for 7.5, 3, or 1 hr. for 5 days/ week. D 11 weeks of exposure with and without Diazepam and alcohol	A	C	-	Math tests	RT
6	Stewart et al.	1977	-	-	-	(ATSDR) 11 week period	A	-	-	-	Coordination
7	Levine et al.	1980	1	-	N	Recycling PER by distillation, excessive heat lead to excessive fumes which killed the subject	A	Case study	-	-	-
8	Stewart et al.	1981	-	-	-	(ATSDR) Acute inhalation at 100 ppm	A		-	-	-

No.	Literature Review of		Tetrachloroethylene (Perchloroethylene)								
			Population				Exposure	Methods	Tests used: Neuropsy		
			N	Ethnic	Control	Description	Acute/ Chronic	Type	Cog.	Memory	Motor, React. Time
	Authors	Year	#	Black White Other	Y/N		A=Acute C=Chron and duration	R=Review C=Clinical E=Epi F=Field	y/n, - indicates no info or no test given	(Attention & Concen.) y/n and list	y/n and rt=reaction time, ft=fingertap
9	Altman et al.	1990	-	-	-	(ATSDR) Acute inhalation at 50 ppm 4 hours for 4 days	A	-	-	-	-
10	Ferroni et al.	1992	-	-	-	(ATSDR) 10 years chronic exposure	C	-	-	-	Reaction Time
11	Gaillard et al.	1995	1	-	N	2 year old male died from exposure from dry-cleaned curtains in his room	A	-	-	-	-
12	Altmanm et al.	1995	19	-	Y	19 of 92 local residents of neighborhoods in Germany with chronic environmental exposure to PER from nearby dry cleaning establishments and 37 matching controls	C 10.6yrs	F	-	-	-
13	Garnier et al.	1996	29	-	-	25 females and five males all cases of acute environmental inhalation exposure between 1989 and 1995. Exposure from large amount of TCE from dry cleaned clothes	A	R	-	-	-
14	Echeverria et al.	1995	4 and 65	-	N	4 Patients were evaluated clinically for neuropsychological deficits in addition to 65 drycleaning employees who were grouped into low, moderate and high exposure groups	C	C & E(F?)	y/n, Trls B,Wisconsin	WMS-R	FT, Santa Ama
15	Ferroni et al.	1992	60women	-	Y	60 Dry Cleaning workers were compared to 30 controls	C	E	SPES: FT, DSy, RT, Vigilance, Shape Compari-son	N	FT

No.	Literature Review of		Tetrachloroethylene (Perchloroethylene)								
			Population				Exposure	Methods	Tests used: Neuropsy		
			N	Ethnic	Control	Description	Acute/ Chronic	Type	Cog.	Memory	Motor, React. Time
	Authors	Year	#	Black White Other	Y/N		A=Acute C=Chron and duration	R=Revie w C=Clinica l E=Epi F=Field	y/n, - indicates no info or no test given	(Attentio n & Concen.) y/n and list	y/n and rt=reaction time, ft=fingertap
16	Gobba et al.	1998	33 dry cleaners, at 12 shops	-	N	33 dry cleaners longititudinal (2 yrs)	C	F	-	-	-
17	Nakatsuka et al.	1992	261 Solvent workers and 120 controls	-	Y	261 solvent workers, 120 controls	C	E	-	-	-
18	Seeber	1989	101dry cleaners and 84 controls	-	Y	101 dry cleaning workers and 84 controls	C	F	Digit Symbol	N	-
19	Schreiber et al.	2002	Residentia l study of families in Dry Cleaner Bldgs.	-	?	6 families living in 2 apartment bldgs. In Nycompared to healthy workers in a day care setting	C	F	-	-	-
20	Cavalleri et al.	1994	35 dry cleaners	-	C	Matched pairs were formed with 35 dry-cleaners in 12 shops and control workes with no exposure	C		-	-	-
21	Spinatonda et al	1994	35 pairs	-	C	39 laundry workers were compared with unexposed control pairs	C	E	Reading tasks	-	-

No.	Literature Review of		Tetrachloroethylene (Perchloroethylene)								
			Population				Exposure	Methods	Tests used: Neuropsy		
			N	Ethnic	Control	Description	Acute/ Chronic	Type	Cog.	Memory	Motor, React. Time
	Authors	Year	#	Black White Other	Y/N		A=Acute C=Chron and duration	R=Revie w C=Clinica l E=Epi F=Field	y/n, - indicates no info or no test given	(Attentio n & Concen.) y/n and list	y/n and rt=reaction time, ft=fingertap
22	Bellinger	2003		-		Theoretical paper on the use of psychological tests which perform as well as medical tests					-

Table 1 (Part B)

No.	Mood		Symptom, Vision, Etc.			RESULTS					Notes
	Dep	Anx	Symp.	Vision	Other	Cog.	Mem.	Motor	Mood	Vis.	
	y/n and BDI, POMS, BSI	y/n and BAI, POMS, BSI	Y/N, list	Y/N, list	list	+/-	+/-	+/-	+/-	+/-	Other relevant info.
1	-	-	Y	-	-	-	-	-	-	-	216 ppm resulted in eye irritation and slight dizziness and inebriation.
2	-	-	Y	-	-	-	-	-	-	-	Semi-comotose state, recovered rapidly
3	-	-	Y	-	Neurological examination, coordination	-	-	-	-	-	25% reported mild frontal headache, small % with Cns Depression. Repeated exposure brought about low objective symptoms. A small percentage had abnormal Rumberg testing.
4	-	-	Y	-	-	-	-	-	-	-	Unconscious but responsive to painful stimuli. Constricted but reactive pupils, left side seizure noted.
5	-	-	Y	-	-	-	-	-	-	-	B: EEG; altered patters suggesting critical depression for those exposed for 7.5 hours to 100 ppm. Coordination decreased with exposure to 100 and 150 ppm. D: Alcohol and diazephram had significant effect on behavioral tests and EEG.
6	-	-	-	-	-	-	-	X	-	-	Observed decrements in coordination at 100 ppm not at 20 ppm.
7	-	-	-	-	-	-	-	-	-	-	Significant levels found in blood and brain at autopsy.
8	-	-	Y	-	-	-	-	-	-	-	EEG changes similar to slight sleep and 1rst stages of anesthesia, also a reduction in overall wave amplitude. Symtoms- headaches, dizziness, and drowsiness.
9	-	-	-	-	-	-	-	-	-	-	Latency of visual- evoked potentials in volunteers.
10	-	-	-	-	-	-	-	X	-	-	Average of 15 ppm for 10 years. Reaction times increased in workers.
11	-	-	-	-	-	-	-	-	-	-	Curtains not properly dried after dry cleaning.
12	-	-	-	Y	-	Neuro-behav. test battery	-	-	-	-	No significant differences between Vigilance, simple reaction time and visual memory.
13	-	-	Y	-	-	-	-	-	-	-	20 cases reported dizziness and drowsiness occasionally headache, nausea and vomiting. 5 had loss of consciousness.
14	1 pt.	4 pts	Y	n	clinical eval.	-	-	-	-	+	Excellent clinical evaluations but lacking visual testing. Low participation rate may influence generalizability.

No.	Mood		Symptom, Vision, Etc.			RESULTS					Notes
	Dep	Anx	Symp.	Vision	Other	Cog.	Mem.	Motor	Mood	Vis.	
	y/n and BDI, POMS, BSI	y/n and BAI, POMS, BSI	Y/N, list	Y/N, list	list	+/-	+/-	+/-	+/-	+/-	Other relevant info.
15	-	-	-	-	epi screening	Y	-	-	-	-	Good exposure measurements but only minimal computrized tests were administered which may not have been sufficiently sensitive to possible impairments.
16	-	-	-	Y	Color vision	-	-	-	-	+	Colorvision evaluation but without visual acuity. Good exposure assessments.
17	-	-	-	Y	Color vision	-	-	-	-	+	6 exposed men were found to have red/green loss.
18	RT	emo-tional ability	neurologi-cal symptoms	-	Digit Reproduction, Digit Symbol and Candellation	-	-	-	+	-	Test Battery probably insufficient but evidence of speed related deficits.
19	-	-	-	Y	-	-	-	-	-	+	Visual Acuity, Contrast Sensitivity and Color Vision findings with good exposure assessments.
20	-					-					
21	-	-	-	-	-	-	RT	#NAME?	-	-	Italian manuscript but translated in Part II. Latencies in speed of reaction were slower for exposed. Dose response relationships were obtained.
22	-	-	-	-	-				-	-	

Review by
Diana Echeverria, Ph.D.

Review by Diana Echeverria

A. General Impressions

- This is a beautifully written document that effectively summarizes enormous disparities in test measures, exposure scenarios, and analyses across human studies. It is a commendable effort.

- I appreciate the clarity of the study summaries. In most cases they were easy to follow against the review I made for each article (See Section C for specific observations). However, where there was good attention on the test measures, their appropriate interpretation, and their potential effect size in the general population as well as on the study population, there was less attention on the epidemiologic issues in study design, constraints of the study population, and common issues associated with categorical data analyses. Several ways that might be implemented is to add a small section or sentence to that effect in the summary of each study. In the synthesis across studies in the later sections, tabulate the variation in analytic approach (chi square, discriminant analyses, anova, manova, multiple regression, adjusted scores etc) followed by summary discussion of why these were used. This will help the reader understand why exposure effect analysis often differ in reported effects from those reported across categorical findings. It helps define the study design.

- I also found it very helpful as I read each study to rate the study on: *Interpretability, Strengths, Weakness, Biological Plausibility,* and *Usefulness to the EPA, i.e.,*
  Spinatonda, et al., 1997
  Interpretability: T-test differences could due to differences in populations and the regression within the exposed group ignores the control group and relies on the performance of a small number of subjects.
  Strengths: Use of a novel test.
  Weakness: Study design does not adequately control for population differences. The sample size is small.
  Biologically Plausible: Given that solvents do increase latencies and response times, it is reasonable to consider this type of test in a test battery. But the current presentation of results is not convincing due to poor presentation and weak study designs.
  Useful for EPA: This is a poorly described small study that found an adverse effect in the correct direction but does not present or describe adequate control for factors that could otherwise explain the effect.

- Include Dick study, 2004

- Add a glossary of test measures**.**

- Why were animal studies not mentioned in the preface (maybe we need an executive summary) and then found located in the back? It is easier to first read about mechanistic

studies which set the frame work of understanding of potential effects in humans. However, in this case there may not be much overlap in functional measures – so is there another way to link the two bodies of information? As it stands, a transition is needed.

B. Charge Questions

Charge a. What are the relative strengths and limitations of the existing human studies of neurological effects of PCE?

Comment: *Sample Size:* The majority of neuropsychological studies, with the exception of Schreiber et al (2002) exceed a smallest cell size of 40 exposed subjects that is generally considered sufficient to detect preclinical effects in a group that range from 3-18 or 20% from normal function. This is true for regression analyses and certainly the power is increased with a simpler matched t-test. However, the
The smaller the population the more reliant one is on subsets of subjects (Cavelleri et al., 1994).

Potential Bias: The most likely source of bias across these cross-sectional studies is subject selection bias. With the exception of those studies that could undertake measurement of PCE exposure or estimate exposure for each individual and use it in a dose-response manner, most studies relied on grouped comparisons between an exposed group and unexposed groups. Authors correctly considered that age and gender are major determinants of exposure and so efforts were made to group match subjects on these factors (Altman, 1995, Cavalleri, et al., 1992; Gobba, et al., 1998). However, Anger et al, (1995) has demonstrated that education is of equal importance which was undertaken by Spinatonda, et al., 1997. There are a subset of studies that did not sufficient control for these factors (Cavalleri, et al., 1992; Gobba, et al., 1998; Schreiber, et al., 2002) and insufficient detail on the study populations was provided (Seeber et al., 2002 (residents), Nakatsuka et al, 1992, Ferroni et., al., 1992, Spinatonda, et al., 1997). There are at least two studies (Cai, 1991; Nakatsuka, 1992) that selected from controls from the same facility. This approach has merit in that self-selctiong factors into that industry are controlled for in the selection process.

There is another source of performance bias that stems from unequal motivation to perform at one's best stemming from a subject's knowledge of a potential risk or from not being blind to exposure status. Schreiber et al., 2002 study design may have introduced such unwanted bias because the study may have been subject to community and parental pressures. Exposure was active among residents and had ceased among day care workers. This scenario might alter motivation. At these very low levels it would have improved the design by evaluating subjects in a blind fashion at a study center. It is noteworthy however, that contrast sensitivity, more so than color discrimination, is not a test subject to very strong motivational effects because it is physiologic in nature.

Ferroni et., al., 1992, is another study that could be affected by uneven motivation. The exposed and unexposed study population of women were tested during the proliferation phase of menstruation (during menstruation), which may better capture changes in prolactin secretion, but also may confound findings if there are individual differences in severity of menstruation and in the timing of the test session relative to the day of menstruation or at least weighted by what day in the monthly cycle. Second, the two groups are from different industries (small shops vs one

hospital) which underscore other likely differences that were not controlled for: operator/owner vs worker, common language, SES and education. Third, the two groups were selected for comparability with respect to age and vocabulary but it is not clear if they were group or individually matched – note the SD of age is +/- 10 years. It is possible that the effect of "duration of exposure" in older more highly exposed operators could be diluted by being mixed in with younger and lower exposed counter and pressor personnel. Fourth, there was no mention of other important selection factors i..e., head injury, diabetes, etc. Other health habits were comparable.

Biological or clinical relevance: Deficits in visuospatial function have been identified in clinical and occupational assessments where it was posited that a frontal/limbic site of pathology might explain the apparent profile of effects documented in patients (Echeverria et al, 1995). In addition, spatially mediated dysfunction has been associated with solvent exposures in industries and even alcohol use itself. In the current set of studies, the primary research question to be answered with respect to visual effects, is whether the effect is localized at the retina (as suggested by Regan, 1989) or more distally on the axon of the optic nerve (Cavelleri et al, 1992; Schreiber et al., 2002; Mergler et al., 1991). The behavioral measures used in occupational studies differ from the more physiologically based measures used in the residential studies in that the former also reflect other CNS function independent of assessing the integrity of the optic and visual nerve function. Therefore evidence from both approaches could provide considerable biological plausibility and clinical relevance that emerges from this review of studies.

Consistency: Overall, there are trends in test measure approaches where occupational studies of workers are more likely to share common tests in their battery. This consistency (Lauwerys et al, 1983; Seeber et al., 1989; Echeverria et al., 1995; Ferroni et al., 1992) is to be expected because selected tests are from common domains recommended by the World Health Organization, the European Union, and ATSDR. The residential studies use more diverse measures and, at least within the set of studies included in this review, are more oriented to using physiological tests such as the Lanthony Color Hue Test or Color Perception Test, or Contrast Sensitivity.

Therefore, with respect to adverse effects on visuospatial function (pattern memory, pattern recognition, Benton, and visual reproductions), one could first confirm adverse findings in visually mediated pathways in high and low exposed populations (Seeber et al., 1989; Altman et al., 1995, and Echeverria et al., 1995). The results across these three studies appear reasonable consistent despite strong differences in study design. The second piece of evidence can be found in both occupational and residential studies (Nakatsuka et al., 1992; Cai et al., 1991; and Cavalleri et al., 1994), all of which evaluated performance on the Lanthony color vision test. Evidence of a trend in the correct direction was established for a high exposed group but not a low exposed group (Cavalleri et al., 1994) among dry cleaners. Adverse effects were also found in a residential population though it did not meet statistical significance. The exception to the trend was the one study by Nakatsuka et al., 1992 that did not find any effect. This study used the Lanthony new color test as a screening tool that was followed by a more diagnostic assessment among those with positive findings using a clinical color vision test. This tiered approach eliminated false positives. It also identified subjects with congenital deficits in color vision in the red-green region. The other concern it that the author applied a Chi ^{2} analysis rather than alternate analytic approaches and therefore may not have good control over covariates. The

available analysis is not conceptually strong and does not use available exposure data to an advantage by weighting area monitoring data with the personal proportion of time spent in the area in a creative manner. For these reasons, this study's negative results must be weighed against the results of the other two studies.

Lastly, at very low levels of exposure that are subject to a very different dose-rate than that incurred by occupational workers, visual contrast sensitivity deficits in PCE-related spatial function but not acuity (a hold test) were also observed (Schreiber et al., 2002). Visual contrast sensitivity is a visual discrimination task assessing the ability to detect a pattern of dark and light bars. Effects appear in the absence of optical, retinal, or optic nerve head pathology. It is a non-specific indicator of sub-clinical visual impairment and sensitive to aging. Therefore, this unique observation is potentially important and should be replicated, particularly at very very low PCE exposures (in the ppb range).

Overall, the evidence argues for a considerable consistency in visually mediated function.

Do the EPA materials adequately evaluate these issues? The strength of this review is in the description of each study, test measures used across studies, and synthesis of findings. The weakness in this review is that the emphasis on examining the results for common threads upon which to build an argument, often over look the issues associated with analytic choices. It would be helpful to expand the description of results and in the summary, the epidemiologic concerns with respect to categorical study designs.

Charge b. How consistent are the visual contrast sensitivity effects seen in one residential study (with two exposed groups) with findings of other visual effects seen in occupational and residential studies where contrast sensitivity was not tested.

Comment: Schreiber et al, 2002, measured visual acuity using a Pocket Vision Screener administered at a standardized distance from the eye. Visual Contrast Sensitivity was manually administered (using the FACT) that is comparable to OPTEC paradigm. The Lanthony (D-15d) detects congenital and acquired discrimination deficits. The reliability of all these tests has been published and can be very high.

The Exposure Assessment is focused on an environmental 24-hour/day dose rate that is considerably lower than all occupational exposures. For residents, the dry cleaner source of exposure was located on the first floor and the range of PCE concentration was established across 16 apartments in 8 NYC buildings. Two were selected that had a range from 650-6,100 ug/m^3 or < 1 ppm (6,800 ug/m^3 =1 ppm so 170,000 = 25 ppm). For the day-care facility, the dry cleaner was located on an adjoining wall. Based on parental complaint, the dry cleaner stopped operating and testing was conducted 6 weeks later.

For the visual analysis the criteria for inclusion was Snellen Equiv of 20:70 or better. This is a good idea. Unit of analysis was the mean of both eyes. Authors applied a MANCOVA using group, spatial frequency, and their interaction term as factors. If global test of significance was met, then VCS scores for each eye were compared to percentiles among controls. Standard use of color discrimination scores for TCDS and CCI is acceptable. Group differences were defined by t-tests for matched pairs of exposed and unexposed subjects. Given this is a small number study – the approach is reasonable. However, in the absence of a D-R analysis, reliance on the selection of controls is very critical in this study. The author could have used metabolites

in urine as an index of exposure in a regression analysis among the residential group to strengthen the resutls.

Acuity was unaffected. VCS was lower among exposed groups. The argument that adverse VCS effects are more likely to be neurologic in origin is biologically plausible. VCS deficits are known to be non-specific indicators of alteration in function. The author correctly states effects appear with glaucoma, macular disease, retinitis, diabetes (Type I), and other distal disease. However, in this case VCS function is more proximal to the visual cortex and therefore can be more readily mediated by adverse CNS function or perturbation of the visual pathway. This is consistent with the occupational studies that report deficits in visually mediated performance.

In contrast, the color discrimination CCI score appeared to differ between exposed and unexposed groups but did not achieve significance. The day care group was analyzed separately and found no differences but an adverse trend was confirmed. The results for color discrimination are remarkable because there was a trend and a visable difference in CCI in the correct direction. This scenario suggests that if the power was greater to detect an effect, one might be able to see an adverse effect at even ppt levels of exposure.

The second piece of evidence is found in both occupational and residential studies (Nakatsuka et al., 1992; Cai et al., 1991; and Cavalleri et al., 1994), all of which evaluated performance on the Lanthony color vision test. Evidence of a trend in the correct direction was established for a high exposed group but not a low exposed group (Cavalleri et al., 1994) among dry cleaners. Adverse effects were also found in a residential population though it did not meet statistical significance. The exception to the trend was the one study by Nakatsuka et al., 1992 that did not find any effect. However, the analysis was not conceptually strong, as described above. For these reasons, I do not put as much weight on the negative results of this study, in contrast to the results of the other two studies.

Lastly, adverse effects on visuospatial function (pattern memory, pattern recognition, Benton, and visual reproductions) in high and low exposed populations (Seeber et al., 1989; Altman et al., 1995, and Echeverria et al., 1995) are reasonably consistent despite strong differences in study design. Collectively, the argument in favor of biological plausibility and consistency is fairly sound.

Charge c. Table 1 summarizes the types of neurologic tests that have been conducted measuring different effects with different populations exposed to PCE. What is the biological and clinical significance of the measured endpoints in these studies?

Comment: *Visual contrast sensitivity* is a visual discrimination task assessing the ability to detect a pattern of dark and light bars. Effects appear in the absence of optical, retinal, or optic nerve head pathology. It is a non-specific indicator of sub-clinical visual impairment and sensitive to aging. It has been found to be sensitive to mercury, solvents, and other neurotoxicants.

Color confusion index is a summary measure of color perception that can distinguish types of errors when ordering colored caps in ascending order to that of colors in the “rainbow.” Red-green deficits are normally congenital. Blue-yellow deficits are thought to be associated with solvent exposure.

Fine motor movement: Hand-eye coordination and finger tapping measure manual coordination and finger speed. The tests were adopted from clinical batteries but have been found to be robust and very useful in conjunction with other measures of motor function (tremor and hand steadiness). Finger tapping is often evaluated with simple reaction time to distinguish finger speed from attention coupled with motor slowing.

Simple Reaction Time: The single most basic human performance test that is a blend of cognitive decision making and motor movement. It is a basic measure of slowed motor response time – the predominant component of the test. If prolonged, the test is also a good measure of attention.

CPT: Is a prolonged attention test often conceptualized as a test of vigilance. It has been found susceptible to solvents, pesticides, anaesthetic gases, and metal exposures.

Visuospatial function: Pattern comparison, pattern memory, and visual reproductions assess the ability to successfully use visual nonverbal material. A pattern comparison require one to compare and identify similarities and differences between patterns in the absence of memory. Pattern memory is comparable but increases the level of difficulty with the addition of recalling unfamiliar shapes that are new to the subject. Similarly, visual reproduction also has a memory component but the test relies on visual perception coupled with fine motor movement of subject and memory to carefully draw pictures. All these tests are sensitive to neurotoxins. Impairment in nonverbal memory diminishes spatial memory, so one can more easily get lost. It also can reduce safety when working with complex new machinery.

Information processing speed: Information processing rates are amenable to establishing the speed at which a person can cognitively process bits of information (msecs/item). Choice reaction time is the most reasonable paradigm in brief assessments because one is only quantifying, at most, a 2 or 4 – choice option. Simple reaction time is usually conducted first, followed in natural progression, with a choice response time where the subject must select and then respond to the correct stimuli. This test was first introduced in the 1950s in laboratory studies assessing basic human performance ability.

Digit Span: This is a test of short –term memory or simple attention. One is measuring the numeric span that a person can recall numbers in the correct order in a forward and backward manner. This test is frequently used in test batteries because of ease of use and interpretation.

Visual scanning task:

Stress Reaction Time:

Trailmaking A and B: This is an executive function test that requires sequencing and alternating sequencing that measure cognitive tracking. This test is sensitive to frontal lobe pathology.

Charge d. What weight should be attached to reported findings of neurological effects in residential populations at exposure levels below those seen in occupational studies?

Comment: This is an important question that does not have an easy answer. Effects are predicated on the selection of subjects in categorical comparisons rather than relying on conventional dose-response. This shift in the analytic paradigm begs the question of using more carefully chosen controls or even multiple control groups (not done), carefully designed test procedures, and different types of tests. In fact, that is what researchers appear to be doing when evaluating physiologic end-points i.e visual contrast, Lanthony, VEPs. Currently, there is not enough redundancy to be confident of ppb –related CNS effects.

The issue of low vs high dose-rate is similar to the argument with respect to elemental mercury health effects in dentists vs those potentially associated with amalgam emissions in teeth. We are clearly going to have to confront this issue straightforwardly by assessing the effects of both modes on the same total amount of exposure.

Charge e. Do epidemiological studies identify susceptible populations? Do the residential studies indicate children and the elderly are more susceptible to the effects of PCE?

Comment: The majority of studies only briefly elude to potential effects elderly subjects above age 60 and there was one pilot study that evaluated Visual Contrast Sensitivity in a small sample of the elderly and children (Fig 1). One can reasonable infer that the risk might be greater in these subgroups because most tests are age-sensitive and the slope or magnitude of effect is more severe. However, this result is a pilot study that requires replication.

Charge f. Do the studies that report decrements in neurological function (including vision) in people exposed to organic solvents add support to the conclusions about the hazards of PCE?

Comment: Yes, there are well described studies among painters, retrogravers, printers, silk screeners, fuel workers, and other chlorinated solvent-exposed populations that clearly report exposure-effect relationships. This evidence increases the plausibility of PCE-related findings particularly over the course of the homologous chain. However, the advantage of PCE studies in dry cleaners, is that the exposure is relatively clean of mixtures so inferences are easier.

Charge g. Can an association be made in the separate studies and in all studies collectively between PCE exposure and observed neurotoxicity?

Comment: There is a natural division is as much as occupational studies predominantly present symptom and neurobehavioral results, where as, residential studies predominantly evaluate physiologic outcomes. But if one accepts the domain of visually-mediated test measures than the argument, interestingly, gets stronger as described above in A and B. I conclude that there is sufficient evidence, using different tests, to support a common mode of action adversely affecting visually mediated function.

Charge h. Are the any published studies or data relevant to neurotoxic risk of PCE which are not included in the discussion paper.

Comment: Yes, I have a large body of solvent manuscripts, only five of which have only been

published in peer-reviewed journals and three others as a BHARC report. Two of those are under peer-review and will be published. All are written – you may have a copy of all of them. Interestingly, in a large PCE study of laundry workers and dry-cleaners, we only confirmed the same result we reported in the 1995 study – three tests of visual spatial function were chronically affected without any acute exposure contribution. What is important is that no other tests were adversely affected.

C. Specific Observations

2. Seeber, 1989

Hypothesis: Do occupational exposures to PCE < 50 ppm adversely affect central nervous system function

Study Design: Cross-sectional.

Methods

Field Study Population: There are two study populations of dry cleaners exposed to low and high exposure groups (n=57 and n= 44). A third population were unexposed sales personnel (n=84). Subjects were not matched on age or gender or education **(See page 9, line 12).**

Test Procedure: All subjects were tested in a 1.5 day stay at a clinic. Test Examiners were blinded.

Behavioral Measures: The test battery is large involving neurological signs, emotional lability, perceptual speed, CRT, Delayed RT, Digit reproduction, Cancellation, and Digit-symbol.

Interpretation: The battery permits differential inferences.

Exposure Assessment: Measurement in air was not well described because they did not have individual exposure data on each person. The only samples described are area samples – a weakness. Low vs High exposure was estimated to be 12 vs 53 ppm.

Analysis: The lack of a linear exposure measure required the team to stratify on age, gender, and intelligence.

Results and Discussion

	Control vs Low	Control Vs High	
neurological signs,	+	-	flat
emotional lability,	+	-	flat
perceptual speed,	+	+	slope
CRT,	+	-	flat
Delayed RT,	(-)	-	step up
Digit reproduction,	+	+	slope
Cancellation,	+	+	slope
Digit-symbol.	+	+	*slope*

Directionality is consistent. Note that this is not a D-R study. But one would expect the difference between control and high subject performance should be greater than control vs low exposed performance.

There was a large difference in exposure between 10 to >50 ppm – need to think about what this means with respect to the other studies. **(page 10, line 14).**

Summary
Interpretability: Very Good.
Strengths: Test battery predicted on clinical observations and frontal/limbic pathology. Symptom profiles discriminated between exposure groups likely due to the use of a control group **(page 10, line 18)** ; no confounding from alcohol.
Weakness: Study design only relies on a dose-response analysis.
Biologically Plausible: The gradient between clinical and pre-clinical effects is plausible if one defines pre-clinical as ~<20% on psychometric tests.
Useful for EPA: This study strengthens the observation that cognitive and psychomotor skills are adversely affected by exposure to PCE. Overall these tests provide other support for an effect but the study did not assess visually mediated functions.

3. Cai, 1991

Hypothesis: Occupational exposure to PCE is associated with increased subjective symptom reporting.

Study Design: Moderately sized cross-sectional study of 56 dry cleaners (27 women and 29 men) from three shops and 37 women and 32 male unexposed controls.

Methods
Study Population: Controls and exposed personnel were from the same facility +/- exposure. The age of male dry cleaners was younger than that of male controls and the age of female exposed women were older than that of female exposed workers.

Test Procedure: The test procedures were not described. **(See page 10 line 35)**

Behavioral Measures: Workers were administered a symptom questionnaire that also was not well described. **(See page 10 line 35)**

Exposure Assessment: 8-hour TWA measurement of PCE exposure was conducted. Mean levels were 20 ppm (4-97 ppm) and mean duration of exposure was 3 years. **(See page 10 line 34**: note that 3 years may not be sufficient to see behavioral effects, but may be enough to see subjective reponses, Echev, 1995).

Analysis: The data analysis of symptoms was not described but it is likely that they are categorical and pool males and females together. If this is true, the study looses specificity where females will report more symptoms, consistent with most solvent studies. **(See page 11 line 1).**

Results and Discussion
The symptoms of dizziness, flushed face, drunken feeling floating sensation are not uncommon in solvent studies. However, it is likely that no effect would have been detected if there had only been an internal analysis within the exposed group. **(See page 11 line 7 – may want to add some conclusion such as this)**

Summary:
Interpretability: Confirmation of consistency between men and women are lost.
Strengths: Demonstrates subjective symptom responses, often omitted in behavioral studies, are useful.
Weakness: Weak description of the analytic plan.
Biologically Plausible: Positive results are consistent with other solvent studies.
Useful for EPA: This study of CNS symptoms does add symptoms to the range of health effects. However, it will not assist EPA in discerning adverse visual system effects from PCE exposure. Exposures are substantially higher than that found in the general population.

4. Nakatsuka, 1992

Hypothesis: Color perception is adversely affected by chronic exposure to PCE.

Study Design: Cross-sectional study among four different exposure groups: Toluene, PCE, PCE and TCE, and a final control group. Genders were unequal across the groups. **(see page 11, line 19)**

Methods
Study Population: The exposed group had 64 dry cleaners (30 males and 34 females) that were compared to a control group of subjects from the same facility but without exposure. An effort was made to match on age but no clear description was provided. **(see page 11, line 34)**

Test Procedure: Testing was undertaken by ophthalmologists and medical doctors and therefore might be conducted in a more proceduralized and clinical manner. However the tests were conducted either under natural sunlight or under a 1150 lux fluorescent lamp. **(see page 11 line 21). It is not clear what procedure was used on what test and when – this could obscure**

potential findings.

Behavioral Measures: The Lanthony new color test was used as a screening tool and this was followed by a more diagnostic assessment using a clinical color vision test. This tiered approach is a very useful assessment approach **eliminating false positives**. **(see page 11 line 32).** Note that this method identified subjects with congenital deficits in color vision in the red-green region.

Interpretation: Blue-yellow color blindness is primarily associated with solvent exposure; red-green color blindness can be distinguished.

Exposure Assessment: Exposures were ~ 18 ppm for dry cleaners.

Analysis: Author's used a Chi ^{2} rather than paired t-tests because they could not match on covariates.
This analysis is not conceptually strong and does not use exposure data to their advantage by weighting area monitoring data with the proportion of time spent in an area in a creative manner.
COMMENT: Consider adding some comment on analytic approach.

Results and Discussion
There was no observed difference based on the Chi Square analysis between exposed and unexposed groups. In fact it appears consistent with the observation that solvent exposure is protective. It is not clear why they did not elect to use their PCE measurement values in a linear dose-response manner in a comparison using exposed PCE workers with controls in the same analysis. Authors postulate that negative findings might be attributable to differences in the solvent type, the intensity of exposure, duration of exposure, or even the presence of peak rather than continuous exposures.

Summary:
Interpretability: The use of four populations is helpful, but interpretation of "protective effect" should be explained.
Strengths: Larger sample size of 64 exposed subjects; increased measurement of effect using a tiered approach; excellent review of the literature.
Weakness: Weak analytic approach.
Biologically Plausible: It is not clear why studies with far lower exposures have found color vision loss and discrimination effects, whereas this one, at higher exposure, is negative. The largest concern is that the analysis is weak for the effect size and study design controls are inadequate.
Useful for EPA: Limited at this time. The data could be restructured by an industrial hygienist and reanalyzed. To be comprehensive one needs positive and negative studies. In this case it is not clear to me whether the negative "effect" is a true negative effect.

5. Ferroni et., al., 1992

Hypothesis: Long-term low-level exposure to PCE may impair dopaminergic control of prolactin secretion and adversely affect behavior. Mechanistically this idea has merit as the perturbation of the neuro-endocrine system maybe one pathway affecting central nervous system function. **(see page 13, line 21)**

Study Design: Moderately sized cross-sectional study of 60 dry cleaners and 30 unexposed controls.

Methods

Study Population: The exposed and unexposed study population of women were tested during the proliferation phase of menstruation (during menstruation), which may better capture changes in prolactin secretion, but also may confound findings if there are individual differences in severity of menstruation and in the timing of the test session relative to the day of menstruation or at least weighted by what day in the monthly cycle. Second, the two groups are from different industries (small shops vs one hospital) which underscores other likely differences that were not controlled for: operator/owner vs worker, common language, SES and education. Third, the two groups were selected for comparability with respect to age and vocabulary but it is not clear if they were group or individually matched – note the SD of age is +/- 10 years. It is possible that the effect of "duration of exposure" in older more highly exposed operators could be diluted by being mixed in with younger and lower exposed counter and pressor personnel. Fourth, there was no mention of other important selection factors i..e., head injury, diabetes, etc. Other health habits were comparable. **(see page 12, line 31)**

Test Procedure: The test sessions were conducted in the morning at respective work sites. Test administrators were not told the intensity of exposure levels.

Behavioral Measures: Workers were administered five "Swedish Performance Evaluation System" tests: finger tapping, simple reaction time, digit symbol, shape comparison-vigilance, and shape comparison-response to stress.

Exposure Assessment: Measurement of PCE exposure was not linked to the (E/ UE) categorical analysis.

Analysis: The categorical data analysis of neurobehavioral effects could be improved because important control for known mediators and confounders were not described or presumed to be evaluated in this preclinical assessment. The directionality of observed effects was good but computing z-scores and conducting t-tests without analytical control for age, premorbid intelligence, test-taking ability, presumes that control by specification is sufficient. **(see page 13, line 8 – might want to add something about role of specification).** In contrast the prolactin study was appropriately thought out. It almost appears that behavior was added on as an after thought and not central to the original study design.

Results and Discussion
Simple reaction time, vigilance, and stress (all based on prolonged response times) response times increased in the exposed group. However, the conclusion stating that "exposure to PCE may significantly impair performance" is only supported by a categorical assessment and is counter to the negative results i.e., "Neither the duration of exposure nor air and blood PCE concentrations were correlated with performance test scores."

Summary: Significant methodologic weaknesses prohibit attributing behavioral deficits to causal exposures. However, the evaluation of prolactin could be significant. The acute association about median air levels of 15 ppm, demonstrated between PCE in blood and air against serum prolactin is likely sound. These results are also consistent with our own parallel findings with acute styrene exposure (Luderer et.al., 2004). **(see page 13, line 11)**

Interpretability: Poor.
Strengths: Demonstrates the dopaminergic system is sensitive to PCE exposure. The results for prolactin stimulate new hypotheses.
Weakness: Poor analytic plan.
Biologically Plausible: It might well be expected to find selective deficits in prolonged response times for visually mediated pathways – the shape-comparison test.
Useful for EPA: This study does not strengthen the collective observation that the visual system is affected by PCE. Exposures are substantially higher than that found in the general population.

6. Cavalleri, et al., 1992; Gobba, et al., 1998

Hypothesis: Color perception is adversely affected by chronic exposure to PCE.

Acute studies show that the visual system is a target function susceptible to change from exposure to PCE toxicity. Specifically, there are deficits associated with VEPs and visual contrast sensitivity. The presence of long-term effects is not well known. An early clinical sign is loss of color vision.

Study Design: Cross-sectional.

Methods
Study Population: The exposed group had 33 female dry cleaners and 2 males in 12 shops. Criteria for selection included: good health history, < 50 g/day ETOH, <30 cigs/day, >= 60% visual acuity with corrective glasses. Criteria for unexposed controls was similar with the addition of no exposure to solvents. The two groups were matched +/- 3 years of age, on +/- 10 g /day ETOH, and +/- 5 cigarettes. COMMENT: There was no matching for education, similar tasks, vocabulary, or test-taking ability, or pre-morbid intelligence. **(see page 14, line 3)**

Test Procedure: Subjects were tested on morning before the exposure assessment at work.

Behavioral Measures: Lanthony D15 Color Hue Test, which is well published, is very susceptible to differences in test administration. For example, Echeverria et al., studies have

never found an effect but have reported tremendous variation in results on the same person if there is stray ambient light in the test environment. Therefore, the test is administered in a black painted and draped box with a specified light bar on top.

Interpretation: Blue-yellow color blindness is primarily associated with solvent exposure; red-green color blindness can be distinguished.

Exposure Assessment: Personal badge (4hrsX2) with CV=2.4-3%. Ambient exposures for operators was ~6 ppm with 8.8 mean years of exposure. This is a very low-level occupational exposure.

Analysis: Multiple regression with control for age, alcohol consumption, (seniority?), smoking, and exposure is acceptable. **(see page 15, line 11- clarify what seniority is)**

Results and Discussion
Differences in the CCI were worse for dry cleaners in comparison with unexposed controls. However, the significant effect on CCI was subtle and not pronounced (1.14 (0.13) vs (1.08 (0.11). Comparisons between exposed operators and exposed pressers were not significant but there were in the correct direction. The subsequent D-R relationship is very dependent on two subjects. Note that the range is 0-11 vs 0-32 in operators. This should be expected. However, Figure 1 clearly shows that only two people fall above 15 ppm or above 1.4 CCI. CCI was not found to be associated with the duration of PCE exposure alone, or the PCE index alone. Therefore, this adverse effect suffers from very few affected subjects and is a weak finding. (**see page 15, line 11 – this is really important to note)**

Summary:
Interpretability: These results over-interpret weak correlations. However, deficits in the blue-yellow range are not directly interpretable as only being the solvent's action on the retina.
Strengths: Exposure is measured and exploratory effects are noted at very low levels of ~6-7 ppm exposure for operators.
Weakness: A broader range of exposure may be needed.
Biologically Plausible: This result has not been replicated by our own and other research studies at higher exposures.
Useful for EPA: The observed effect is not rapidly reversible.

Gobba, et al., 1998

Hypothesis: Deficits in Color perception are reversible.

A follow-up study on the same population was conducted two years later.

Study Design: Longitudinal.

Methods
Study Population: This is the same group but two subjects had retired.

Test Procedure:
Behavioral Measures: Lanthony D15 Color Hue Test coupled with a survey.

Exposure Assessment: Personal badges were used and ambient exposures increased in a subset of subjects from 1.67 ppm to 4.35 ppm, and had decreased in another set from 2.95 ppm to .66 ppm. The overall mean was basically unchanged ~1.94 vs 2.4 ppm. These are very low occupational exposures.

Analysis: ANOVA was adopted with control for age.

Results and Discussion
Overall, CCI losses increased with age (r=.45) and with increasing exposure to PCE in the higher exposed sub group (r=.39). Effects in the group that experienced reduced exposure over the two-year time period did not experience more losses in color perception. Deficits remained in the blue-yellow region – consistent with the literature that has found positive effects.

Summary:
Interpretability: The follow-up results improve interpretation above and beyond the original over-interpretation in the original study. (**see page 15, line 5)** However, deficits in the blue-yellow range could be interpreted either as localized action on the retina or as a more distal effect on an axon of the optic nerve.
Strengths: Exposure is measured CCI is measures prospectively.
Weakness: A broader range of exposure may be needed. (**see page 16, line 3)**
Biologically Plausible: This result has not been replicated by our own and other research studies at higher exposures.
Useful for EPA: The observed effect is not rapidly reversible.

7. Echeverria, 1995

Hypothesis: Occupational exposure to PCE adversely affects central nervous system function on a possible continuum between clinical and pre-clinical effects.

Study Design: A large clinical assessment among four patients diagnosed with PCE neuropathy exhibited deficits in frontal lobe and limbic function – a proposed site of pathology. Consistent with this hypothesis, long-term deficits in visuospatial skill, memory, and disturbances in mood were also evaluated in 65 dry cleaners in a cross-sectional assessment.

Methods
Field Study Population: There were no controls. Instead the study relied on generating a linear dose-response curve among a group of dry cleaners with a wide range of measured exposure. Criteria for inclusion: spoke English, > 1 year in a shop, no history of CNS problems. Subjects in the high exposed group of operators were group matched in age, gender, and education with lower exposed pressors and counter personnel.

Test Procedure: All subjects were tested in the beginning of the week (Monday/Tuesday) in a

van after work at the facility.
Behavioral Measures: Given the hypothesis that memory for visuospatial material should be affected and tests of simple attention and basic verbal skill should affected to a lesser extent, the NES test battery included mood, symbol digit, digit span, trailmaking A/B, visual reproductions, pattern memory and pattern recognition.

Interpretation: The battery permits differential inferences where the last three test primarily assess visually mediated function.

Exposure Assessment: Measurement in air and in breath were generated from a robust relationship between breath and air. This permitted one to only measure the concentration in breath and take multiple ambient air levels for each subject as a series of grab samples on the day of testing.

Analysis: Appropriate use of multiple regression with adequate control for current exposure, sub-chronic <3 years of exposure, and chronic cumulative exposure, against age, gender, education, vocabulary, and alcohol consumption, but note that the study examiners were not blinded.

Results and Discussion
Only the three tests of visually mediated function were adversely affected by chronic exposure, consistent with that found among patients. Unexpectantly, there were few symptoms and no adverse mood effects, attributable to either low exposure or the absence of a control group. **(See page 18, line 12- should add this sentence somewhere here).**

Summary
Interpretability: Very Good.
Strengths: Test battery predicted on clinical observations and frontal/limbic pathology.
Weakness: Study design only relies on a dose-response analysis; not blinded; small sample; not control group precluding general population inferences.
Biologically Plausible: The gradient between clinical and pre-clinical effects is plausible if one defines pre-clinical as ~<20% on psychometric tests.
Useful for EPA: This study strengthens the observation that the visual system is affected by PCE but the exposures are substantially higher than that found in the general population.

8. Altman, 1995

Hypothesis: Residents living in potentially exposed neighborhoods exposed to Tetrachloroethylene experience adverse neurophysiologic and neurobehavioral central nervous system function.

Study Design: This is another small cross-section assessment (E=19/UE = 30). Subjects were age and gender matched. Education and vocabulary may not be as important with respect to VEPs unless one is concerned with following good instruction. However the other behavioral tests warrant control for premorbid intelligence in the comparison group (**see page 19, line 15)**. Clinical screening for other health status prior to conducting the assessment strengthens the

findings.

Methods
Study Population: Controls were matched on age and gender. Criteria for exposed: blood > 2 ug/l, >=1 year of exposure, and no other occupational or hobby-related solvent exposure.

Test Procedure: Subjects were administered an extensive test battery. A clinical exam screened out hypertension, endocrine disorders, and hand wrist disorders – a strength of the study design. (**see page 19, line 22)**. Education levels differed in the exposed group (E= 4/8/2 vs UE=1/12/10) indicating a need to analytically control for test-taking ability and basic skill. (**see page 19, line 26)**.

Behavioral Measure: Pattern reversal VEP is a widely used physiologic measure applicable to clinical and pre-clinical assessments. Vibration perception was administered to rule out peripheral insult (a crude assessment) using a tuning fork. Five German equivalents of the NES finger tapping, hand-eye coordination, continuous performance test , simple reaction time, and visual memory (Benton visual retention test) were administered in one 3-hour test session.

Interpretation: The pattern reversal VEP has an advantage over flash-evoked VEPs. The measure usually has smaller "within and across subject" variability in normal groups; is more reliable; and provides information on the integrity of the visual system. The author considers the increased reliability to afford more physiologic sensitivity to a neurotoxic exposure. This argument should be balanced by whether the measure can vary with subtle low level exposures and whether physiologic effects appear first or follow CNS ones. (**see page 20, line 26)**. The NES is a validated test battery used in many solvent assessments.

Exposure Assessment: A blood sample was drawn twice, one in the apartment and once on the day of the exam. As this is a continuous low level 24 hour ambient exposure, one would expect part per trillion exposure levels. All exposures are very low but there were magnitudes of difference observed between exposed and control group air levels (.7 ppm vs .0005 ppm). Blood levels would be expected to have even smaller differences attributable scaling and being in a biologic media (.0178 mg/l vs .0005 mg/l). The correlation between blood and air levels was excellent in the apartment and lower in the exam room (~.81 vs .24) suggesting that removal from exposure during testing could only measure a chronic effect?. (**see page 20, line 16)**. COMMENT: It is not clear to me how such a small difference matters in comparison to the effect of subject selection.

Analysis: Appropriate use of multiple regression with adequate control for age, gender, and education but note that the study examiners were not blinded.

Results and Discussion
The integrity of the visual system was not adversely affected by exposure but visual memory was suggesting that the perturbation is somewhere along the visual pathway. Finger speed and hand-eye coordination were not affected, but SRT was. This profile might suggest the cognitive component of SRT, associated with prolonged attention, may be more susceptible. This observation is consistent with deficits in the latency of visual memory. (**see page 20, line 21)**.

Summary
Interpretability: Very Good.
Strengths: Sound study design and thoughtful test battery.
Weakness: Study design precludes dose-response analyses; not blinded; small sample.
Biologically Plausible: The visual memory result is within a pre-clinical range, appears plausible, because researchers have repeatedly shown that solvents do perturb visual function, and do discriminate between the physiologic system and the centrally mediated behavioral response.
Useful for EPA: This study strengthens the observation that a low but consistent dose-rates might affect visual memory but not the integrity of the visual system.

9. Spinatonda, et al., 1997

Hypothesis: Vocal reaction time (VRT) is adverse affected by PCE exposure among dry-cleaners

Study Design: This is moderately sized cross-sectional study (E=35/UE=39). Characterization of the population is unclear. Both acute and chronic effects were evaluated using acute exposure and a cumulative exposure index.

Methods
Study Population: Controls were matched on age and education (a surrogate for pre-morbid intelligence). However there was no information provided on the characteristics of both groups. Therefore the quality of comparisons is not clear.

Test Procedure: Test environment differed. Testers were not blinded to exposure status. Workers were tested at their work-place and the control group was tested in a research laboratory. This inequity may impact findings. (**see page 21, line 14- equally important)**.

Behavioral Measure: Speech production may be a useful assessment for early detection of CNS sub-clinical effects. This test is not used routinely or frequently in solvent studies. The latency and duration of a vocal response was measured after reading concrete and nonsense words on a screen. There was an additional level of difficulty by requesting subjects to respond immediately or following a delay of .1 or 1.5 seconds. The reliability of this test is not reported.

Interpretation: This is a verbal reaction time test that is likely sensitive to the chronic effect of age. Gender differences in verbal latencies and durations may be comparable to that of SRT. It should also be sensitive to alcohol. The author justifies this test on the basis of sensitivity at preclinical phases of effect e.g., "Activation (attributable to PCE) of complex functions at an initial stage (pre-clinical) can identify dysfunction."

Exposure Assessment: Grab samples defined acute exposure which is not necessarily a weakness if attention is paid to homogenous exposure zones. The Cumulative indices of exposure were computed. However, the duration of exposure was not described.

Analysis: T-tests were used to determine health effects between exposed and unexposed groups. Authors assumed selection on matched age and education is sufficient. A stronger analysis could

have been performed using regression with the entire population of exposed and unexposed, controlling for age, education, and alcohol. The latter options was not discussed. Given the lack of information on the impact of individual differences between the groups, the t-test analysis is weak and inconclusive. The regression model within the exposed is better but ignores the control group. Also the use of sub groups is always problematic in small studies. The nonlinear analysis does not address weakness. (**see page 21, line 34 – this is an important observation about this study)**.

Results and Discussion
The authors' dependence on differences on t-tests is over-interpreted. The differences between groups could reflect their familiarity or lack of familiarity with "concrete" words, which is not an issue with the meaningless words. Also the regression results within the exposed group is driven by three subjects above 600 ms above 50 on the exposure index.

Summary
Interpretability: T-test differences could due to differences in populations and the regression within the exposed group ignores the control group and relies on the performance of a small number of subjects.
Strengths: Use of a novel test.
Weakness: Study design does not adequately control for population differences. The sample size is small.
Biologically Plausible: Given that solvents do increase latencies and response times, it is reasonable to consider this type of test in a test battery. But the current presentation of results is not convincing due to poor presentation and weak study designs.
Useful for EPA: This is a poorly described small study that found an adverse effect in the correct direction but does not present or describe adequate control for factors that could otherwise explain the effect.
10. Schreiber, et al., 2002

Based on evidence from higher exposed occupational studies and from indoor-air low exposure studies, adverse effects are selective for visual system processes or processes dependent on rapid detections and information processing.

Hypothesis: Residents in apartment buildings and day care facilities that reside in close proximity to dry cleaning establishments inside their buildings, experience adverse visual function effects attributable to exposure to PCE.

Study Design: This is a small cross-sectional visual function assessment. The exposed and control populations might introduce unwanted variance from individual differences. Testers were not blind to exposure status. Exposure was active among residents and had ceased among day care workers. This scenario might alter motivation. At these very low levels it would have been better to evaluate subjects at a study center. If community awareness was heightened, motivation to perform well might be reflected in test scores. (**see page 23, line 15)**. However, contrast sensitivity, less so than color discrimination, is not a test subject to very strong motivational effects or education. Further, test administrator involvement can be further reduced by

implementing the visual acuity test and contrast sensitivity test in a standardized apparatus.

Methods

Study Population 1. Residents (E=17/UE=25). Families in two building were contacted and enrolled if they lived in the building for >1 year; volunteered to permit an exposure assessment (badges in apartments), provide biologic samples, complete a visual test battery, and complete a questionnaire. *Seventeen exposed subjects from six families were enrolled*. *Twenty-five* age and sex matched controls were recruited from NYSDOH workers and their children in Albany.

COMMENT: Authors might have considered matching controls on education, skill level such as white or blue collar job, vocabulary, or SES that could vary between locations. (**see page 22, line 18)**. This is a very small group of subjects.

Study Population 2. Day-care facility employees (E=9/UE=9). One facility in one building was contacted after the dry cleaner ceased operations. All nine female staff members participated and were age and gender matched with nine friend controls.

COMMENT: A friend control is interesting and might be an improvement over the resident control criteria. However, authors might have considered matching controls on education, type of job, vocabulary, or SES that could vary between groups. Again, the effect size in very small groups of subjects will vary with the selection of a control group. (**see page 22, line 25)**.

Test Procedure: It is not clear where the extensive test battery was administered. (**see page 23, line 10)**. This is at least a three hour assessment and one presumes it was done at a study center. However, the absence or presence of PCE on the day and time of testing was not defined for the residents. The day care investigation was conducted six weeks post-closure so acute exposure may not be a concern but standardization of test stations and test location is a concern.

COMMENT: The publicity status of this study may be a factor. Was this study publicized? Motivation to perform could be affected by community and parental concerns, particularly in a day-care facility. (**see page 24, line 23 – also applicable to children as well as adults)**.

Behavioral Measures: Visual acuity was measured using a Pocket Vision Screener administered at a standardized distance from the eye. COMMENT: OPTEC has better control for a test-environment. PCE should not affect acuity so I would think this is a hold test even though it was not defined in an a priori manner.

Visual Contrast Sensitivity: Authors used a manually administered test (FACT) that is comparable to OPTEC paradigm.

Color discrimination: Lanthony (D-15d) detects congenital and acquired discrimination deficits. This test is subject to variation in results associated with ambient lighting. The use of a lamp without ambient shielding may compromise results.

COMMENT: Our studies used a portable test “black box” equipped with the same lamp. Stray ambient light significantly altered the outcome (Echeverria, 1994). (**see page 25, line 30)**.

The reliability of all these tests has been published and can be very high.

Interpretation: Visual acuity is a hold test. Visual contrast sensitivity is a visual discrimination task assessing the ability to detect a pattern of dark and light bars. Effects appear

in the absence of optical, retinal, or optic nerve head pathology. It is a non-specific indicator of sub-clinical visual impairment and sensitive to aging. No known differences between men and women. Color discrimination was used as an indicator of effect test.

Exposure Assessment: The research concern is with respect to an environmental **24-hour/day low-dose rate** that is considerably lower than all occupational exposures. For residents, the dry cleaner is located on the first floor and the range of PCE concentration was established across 16 apartments in 8 NYC buildings. Two were selected that had a range from 650-6,100 ug/m^3 or < 1 ppm (6,800 ug/m^3 =1 ppm so 170,000 = 25 ppm). For the day-care facility, the dry cleaner was located on an adjoining wall. Based on parental complaint, the dry cleaner stopped operating and testing was conducted 6 weeks later.

Residential subjects provided a first morning void urine sample (for TCA and TCErOH metabolites) and a breath sample (half-life of PCE in breath is ~2.5 hours so this may be reasonable). Subjects >17 years of age also provided a blood sample. Breast milk was collected on lactating mothers. Personal samples (slightly lower) and area samples were collected with means of .35, .18, .14 ppm air levels. Exposure was not collected on day-care workers.

Analysis: For the visual analysis the criteria for inclusion was Snellen Equiv of 20:70 or better. This is a good idea. Unit of analysis was the mean of both eyes. Authors applied a MANCOVA using group, spatial frequency, and their interaction term as factors. If global test of significance was met, then VCS scores for each eye were compared to percentiles among controls. Standard use of color discrimination scores for TCDS and CCI is acceptable. Group differences were defined by t-tests for matched pairs of exposed and unexposed subjects. Given this is a small number study – the approach is reasonable. However, in the absence of a D-R analysis, reliance on the selection of controls is very critical in this study. COMMENT: Authors could have used metabolites in urine as an index of exposure in a regression analysis among the residential group.

Results

Study Population and Exposure: Age, gender, other exposure to PCE, and alcohol consumption was equivalent in both exposed and control groups (with the exception of one person). Mean duration in the apartment was ~5 years. Mean duration at work in exposed building was equivalent to ~4 years (but exposure was intermittent like an occupational group). Exposure in air was measured before and after dry cleaning closure. The drop in exposure was significant in some places (82%). Breath concentrations were correlated with air levels and (r=.91) and were above standards. Due to the long half life and very low exposure, correlations between urine and blood, breath, and air levels were variable and lower. Urine metabolites are not chronic indicators of exposure.

Vision: Acuity was unaffected. VCS was lower among exposed groups. Color discrimination CCI score appeared to differ between exposed and unexposed groups but did not achieve significance. The day care group was analyzed separately and found no differences. An adverse trend was confirmed.

COMMENT: These results suggest visual contrast is a good indicator of low-level toxicity. The results for color discrimination are remarkable because there was a trend and a visable difference in CCI in the correct direction. This scenario suggests that if the power was greater to detect an effect, one might be able to see an adverse effect at even ppt levels of exposure. (**see page 25, line 30)**.

Discussion

Visual System Conclusions: This reviewer agrees that acuity is sound indicating that optical refraction or the ability of the eye to focus images on the retina is intact. Further the argument that adverse VCS effect is more likely neurologic in origin is biologically plausible. VCS deficits are known to be non-specific indicators of alteration in function. The author correctly states effects appear with glaucoma, macular disease, retinitis, diabetes (Type I), and other distal disease. However, in this case VCS function is more proximal to the visual cortex and therefore can be more readily mediated by adverse CNS function or perturbation of the visual pathway. For example, adverse VCS effects are associated with chronic aging, optic nerve neuropathy, optic nerve compression, and cerebral lesions. Therefore, conceptually, the argument is reasonable.

The interpretation of negative color discrimination effects is not as clear. It is our experience that this particular test (Lanthony D-15) is subject to variation in distinct test environments. Therefore, the fact that it was not as sensitive to low PCE effects may be due to low exposure, differences in dose-rate between residential and occupational studies, or test environment. It is a more complex test than VCS and therefore test-strategy may make a difference, which in turn, is subject to motivation. (**see page 25, line 31- think about it)**.

Summary
Interpretability: Good.
Strengths: Used three tests of visual function in two populations with a hold test.
Weakness: Study design did not control for motivation in the study design. There might be some test-taking bias.
Biologically Plausible: These exposures are very very low. The subtly pre-clinical results do not uniformly implicate deficits in visual function but the results with VCS might suggest some persistence given an exposed group and a formerly exposed group both performed differently than the exposed group. Color discrimination, known to be more variable, is not as sensitive to exposure as VCS. Individual differences in performance could not be evaluated given the size of this group. Overall, this reviewer can not exclude the possibility that the adverse visual effect may reflect damage or impairment in other parts of the brain cortex.
Useful for EPA: This is a very good small numbers study that requires replication. The study design precludes evaluating special susceptibility is the aged or children. However, the results in contrast sensitivity are plausible and are strong enough to warrant a more comprehensive assessment.

Review by
Fabriziomaria Gobba, M.D.

Review by Fabriziomaria Gobba

GENERAL COMMENTS

A preliminary comment is that the "Discussion Paper on Neurotoxicity of PERC" is strictly limited to the effect of this solvent on nervous system, while aspects necessary to understand the health effects (including neurotoxic effect), as the toxicokinetics and the factors influencing toxicokinetics, indices for the biological monitoring and their limits, etc. are not included. I suppose these aspects are adequately addressed in other parts of the EPA document on PERC, as it is absolutely clear that, just as an example, a knowledge of PERC toxicokinetics is needed to understand the difference, in terms of internal dose and potential toxicity, between occupational and environmental exposure.

The document presents an extensive review of literature data on neurotoxic effects of perchloroethylene (PERC). Human studies are mainly considered and discussed, but a body of relevant data from animal studies are also included. More emphasis is addressed to human studies, compared to animal, but I think this is correct, as comparability of results obtained in humans and in animals is far than complete.
On my opinion the review is really well done, and present correctly information. The large majority of relevant papers on PERC neurotoxicity published in English are included and commented.
Possibly, in the part where individual papers are presented, some more comment on the limitations of the study may be included.
Furthermore, I suggest to consided in the review some studies not included. A list is reported in the Charge Questions, letter h of the comments sent before of the Washington Meeting. Some papers are in languages other than English.
More specifically, I suggest to include a paper of Onofrj M et al (Clin Toxicol 1998; 36:603-607) describing a case of clinical optic neuritis in a dry-cleaner. Onofrj et al published another paper (Optic neuritis with residual tunnel vision in perchloroethylene toxicity) in European Neurology (1999;41:51-53) but, apparently, it is another description of the same case.
I suggest to consider also the papers of Mirzoev and Sultanov (Oftalmol Zh (Odessa). 1989;5: 262-65) and of Takeuchi et al. (Jap J Ind Health. 1978;20:146-155) reporting electrophysiological and other eye effects in PERC exposed workers. In the paper of Mirzoev et al. an effect on ERG was observed also in animals.
Another paper eligible for inclusion is that of Alieva et al (Gig Trud Prof Zabol 1985;2:11-13), reporting an increased prevalence of dyschromatopsia in workers exposed to Styrene and PERC (environmental level: 20 mg/m3).
The reason to include in the review these papers, even if they are written in Russian (Mirzoev et al; Alieva et al) or in Japanese (Takeuchi et al), causing some difficulties in their interpretation, is related to the fact that they describe electrophysiological objective effects and clinical effects to the eye in workers exposed to PERC (in the review, only sub-clinical and subjective eye effects -as colour vision loss or VCS deficit- are presented).
As stated in the review, both colour discrimination loss and VCS reduction may be an early sign (or, possibly, the only sign) of pathology in the retina or optic nerve.

Accordingly,
1) if PERC can induce a clinical pathology in the optic nerve
and
2) if colour vision loss or VCS loss may be early signs (possibly the first) of clinical pathology, it is reasonable to hypothesize that the loss in colour vision and/or the reduction in VCS repeatedly observed in PERC exposed workers represent the first (possibly the only) sign of pathology induced by the solvent to the eye The observation that colour vision loss is dose-related (Cavalleri et al. 1994), that an increase in PERC exposure may induce a progression of the impairment (Gobba et al, 1998), and that a (tendential) loss in colour vision may be observed togheter with VCS in PERC environmental exposure (Shreiber et al) further support the hypothesis.

Another observation deserving inclusion in the discussion is that an impairment in colour vision in PERC exposed subjects was observed by 3 different research groups:

1. Cavalleri et al (1994) in workers; the result was further confirmed in the same workers in a follow up performed two years later by Gobba et al (1998); both studies are from the same research group;
2. Alieva et al (1985) in workers;
3. Schreiber et al (2002) in subjects exposed to low environmental levels of PERC (even if the result is not significant, possibly related to low exposure or/and to the relatively small number of subjects tested).

The only one non-positive study on colour vision in PERC exposed workers was published by Nakatsuka et al (1992), but these authors applied the Lanthony's new colour test, that is less sensitive compared to the Lanthony's Desaturated 5 Hue Test for early detection of colour discrimination deficits (Geller and Hudnell 1997);
Furthermore:
- the results of the test were evaluated qualitatively and not quantitatively (e.g. using the methods proposed by Bowman, 1982);
- testing conditions were not adequately standardized: a) tests were carried out by "ophthalmologists or occupational health doctors", suggesting that different group of workers were tested by different persons; b) different lighting conditions were adopted: natural sunlight or "daylight" fluorescent lamp;
- exposed and controls were not matched for age;
- alcohol consumption was not considered.
Accordingly, an evaluation of the results of Nakatsuka et al. is problematic.

The results obtained by Schreiber et al (2002) and, possibly, other studies in workers exposed to different solvents (e.g. solvent mixtures, Broadwell et al, 1995) suggest that contrast sensitivity may be more sensitive compared to colour vision. Nevertheless but it must be considered that comparisons between VCS testing and colour vision testing in solvent exposed subjects are limited, and further good quality studies in this field are certainly need.

As correctly stated in the Preliminary Document, as a whole, the results of studies in PERC exposed workers and that in experimental exposure in animals both support the hypothesis that the nervous system is the critical organ for PERC inhalational exposure in humans. An

increasing body of data suggest that tasks requiring visual information processing, as colour vision, VCS, and some neurobehavioural tests may be affected at low (and also at very low) PERC exposure levels. A possible conclusion is that visual function is particularly vulnerable to this solvent.
Nevertheless, I suggest to explicit in the Doc the difficulties related to the fact that the pathogenetic mechanism(s) is (are) unknown. A peripheral (retinal?) location of the effect is suggested by some electrophysiological data and also by the type of colour vision defect observed in workers, according to the "Kollner's rule" (nevertheless, exceptions to the "Kollner's rule are known).
On the other hand, some studies, including that of Dick et al (2003), suggest another possibility, i.e. that the effect on vision may be the result of a more generalized effect on nervous system. According to this hypothesis, an effect on visual function was observed because tests applied to study visual function (VCS and colour vision) are (possibly) more sensitive compared to other neurobehavioural tests currently applied.
Unfortunately, scientific data on this aspect are largely insufficient, and there is no agreement among researchers (including Peer Reviewers of the PERC EPA Document).

There are also other studies to be considered for inclusion in the review, as that of Seppalainen and Antti Poika (1983), Lindstrom et al, (Neurobehav-Toxicol-Teratol. 1982 Sep-Oct; 4(5): 581-8) and of Antti-Poika,-M (Int-Arch-Occup-Environ-Health. 1982; 51(1): 81-9, and 1982; 51(2): 127-38), suggesting that PERC may be among solvents inducing the "chronic organic solvent intoxication".
Some further studies, all from Polish researchers (Sinczuk-Walczak et al, Polakowska et al) suggest that occupational PERC exposure may affect EEGs. An evaluation of these studies is difficult, mainly due to language, but the results are coherent with data reported in controlled inhalational exposure by Stewart et al (1997).
Lastly, a paper of Muttray et al (Dtsch-Med-Wochenschr. 1999 Mar 12; 124(10): 279-81) suggests that PERC exposure may be a (rare) cause for sleep apnoea syndrome.

CHARGE QUESTIONS

Charge a. Limitations more frequently observed in human studies are:

- the number of subjects included relatively small: from 19 to 65 in all but one papers on chronic exposure included in the Discussion Paper; several factor may affect the nervous system: the control may be problematic if small groups are considered;
- the selection of controls, e.g. in some studies results may be influenced by difference in cultural level and/or in motivation between PERC exposed and controls ;
- the evaluation of PERC exposure: as the relation between external and internal dose may vary, at least on an individual basis biological monitoring (e.g. PERC in blood or in alveolar air) may more representative of effective exposure (= internal dose) compare to environmental monitoring; curiously, even if several authors measured both PERC in air and in blood (and/or in alveolar air), in most studies environmental data are mainly considered;

- there is an high variability of the protocols applied in different studies, limiting the possibility of comparison.

In the Discussion Paper, limitations are briefly introduced in the Review of Individual Studies (1.1), while is in the Summary (1.2, pag. 26-29) that main limitations are discussed.

Some further comments:

Lauwerys et al, 1983: exposure was evaluated by active personal sampling, that was a common method in 1983; some further results obtained using passive monitoring are reasonably comparable. The description of the method applied for alveolar sampling is poorly described. An observation on this paper is that the correlation between environmental concentrations of PERC and values of biological indices was not significant: this raise a question on the correct evaluation of exposure, at least on an individual basis.
Another observation is that **critical fliker fusion (CFF) significantly differed between exposed and referents, but this difference was apparently neglected by Authors**.

Nakatsuka et al, 1992: the limitations of this study have been presented in the General Comments.

Echeverria et al, 1995: in the field study, only 23 of 125 dry-cleaning shops agreed to participate: a selection bias cannot be excluded.

Altmann et al, 1995: the controls were recruited from the staff of a Public Health Office or an Institute for Environmental Hygiene; on our experience this may introduce difference in motivation compared to workers, possibly influencing the result of tests.

Schreiber et al, 2002: evaluation of exposure is problematic as the activity of dry-cleaning shops was interrupted some while before testing of visual function.

Charge b. The main problem in answering to this question is related to the lack of knowledge on pathogenesis: we have no sound scientific data on a possible common pathogenetic mechanism (or possible pathogenetic mechanisms) .
Nevertheless, the facts are:

- different studies (colour vision testing, neurobehavioural tests) performed by different research groups show that visual function may be affected in subjects with chronic inhalational PERC exposure;
- VCS testing is considered a sensitive test for early detection of pathology to the visual system (Jindra and Zemon, 1989).

In the absence of studies supporting different explanation, it is rationale to hypothesize that the VCS effect observed in the study of Schreiber represents **an early manifestation of neurotoxicity of PERC to the visual system**. Note that the trend toward worse colour discrimination in the same group, even if not significant, is coherent with this hypothesis.
As a conclusion, the effect on VCS can be considered consistent with other visual effects seen in other studies, even if no scientific data specifically supporting this conclusion are available to

date.

Charge c. In Table 1 the effects on nervous system observed in studies in PERC exposed subjects are presented; various endpoints are included. The most part of relevant studies presented in peer reviewed papers are included, even if some more papers can be included (a list was presented during the Meeting). The results presented are significant in the sense thata they give a coherent body of data showing that nervous system is a relevant target organ in PERC inhalational toxicity.

Charge d. Excluding the results of Altmann et al (1995) and Schreiber et al (2002), in the studies included in Table 1 the LOAEL for chronic inhalational exposure is between 4 ppm (Gobba et al, 1998) and 41 ppm (Echeverria et al, 1995), with the most part of studies suggesting a LOAEL between 7 and 15 ppm (Cavalleri et al, 1994; Ferroni et al, 1992; Seeber et al. 1989; and Spinatonda et al 1997). It can be observed here that these values are substantially coherent with the LOAEL obtained in studies on hepatic toxicity (Brodkin et al, 1995) and nephrotoxicity (Mutti et al, 1992).
A relevant exception are the results reported by Altmann et al (1995) and, more recently, by Schreiber et al (2002) suggesting a LOAEL for the effect of PERC on nervous system much lower, in the order of 0.2 ppm, considering median values.
Even if some aspects of both studies can be discussed (e.g. small number of subjects included or selection of referents), and even if replication of results is certainly needed, we cannot ignore that **the only two published papers on environmental exposed groups both show an effect on nervous system at a similar environmental level of PERC, much lower (1 order- 2 orders of magnitude) compared to all the other studies** (all performed in exposed workers).
An important point here is if the difference of the LOEAL observed in studies in environmental exposure vs. studies in occupational exposure can be explained.
An obvious possible reason is that workers are exposed for 8 hours/day for 5 days/week for max 40 years, excluding periods sick leave, etc., while **people exposed due to contamination of their houses are likely to be exposed for 12-16 h/day, for 7 days/week, for 12 months/year**: of course this is much different in term both of internal dose (longer periods of exposure = increase in absorbed dose at the same environmental level) and of kinetics of exposure (shorter periods without exposure).
Accordingly, the results of studies of Altmann and Schreiber cannot be ignored, even if certainly they deserve replication (or non-replication).

Charge e. The number of subjects examined by both Altmann et al (1995) and Schreiber et al (2002) is relatively small to evidence factors of increased susceptibility in sub-groups. Nevertheless, in the study of Schreiber et al. 4 children and 2 older than 60 y were included: the authors conclude that results are indicative of an increased susceptibility to PERC.
Children, aged people and pregnant women are usually considered sub-groups with an higher susceptibity to solvent toxicity but, obviously, they are not included in the studies on occupational PERC exposure. Nevertheless, an increased susceptibility to PERC neurotoxicity during the latter part of pregnancy and early life was suggested in studies on reproductive and developmental susceptibility (Beliles RP. Concordance across species in the reproductive and developmental toxicity of tetrachloroethyene. Toxicol Ind Health 2002;18:91-106). Furthermore,

abnormal chromatic responses and reduced contrast sensitivity was described in a 2,5 year-old boy following prenatal exposure to PERC (Till et al. Assessment of visual functions following prenatal exposure to organic solvents. Neurotoxicology 2003;24:725-31). Similar results were also described in women exposed to other organic solvents (Till et al, 2001).
Accordingly, some **data do exist suggesting an increased susceptibility to PERC at least during pregnancy and early life**.

Charge f. The pathogenetic mechanism(s) of decrement in visual function in PERC exposed subjects (and, more generally speaking, exposed to organic solvents) is (are) unknown, and the same is valid also for the other neurobehavioural effects. Accordingly, the answer is only speculative.
Nevertheless, taken as a whole, the studies consistently indicate that PERC exposure may induce a neurotoxic effect. Possibly, this effect may be induced at (very) low exposure level.
Accordingly, **the set of studies as a whole indicate that PERC exposure is a potential heath hazard to the general population**.
Nevertheless, stronger scientific basis to support this assumption are opportune, and this field certainly deserve further development.

Charge h. *(As in previous comments).*
Here you can find a list of relevant papers. The abstract of some papers is also enclosed.

Onofrj M, Thomas A, Paci C and Rotilio D. Optic Neuritis with residual tunnel vision in perchloroethylene toxicity.
Clin Toxicol 1998; 36:603-607.

Mirzoev TA, Sultanov MY. The action of styrole and tetrachlorethylene on electric activity of the retina.
Oftalmol Zh (Odessa). 1989;5: 262-65 (in Russian, English Abstract);

Takeuchi Y, Hisanaga N, Koike Y, Mabuchi C. Two cases presumably poisoned by perchloroethylene in Japanese-style silk clothes cleaning.
Jap J Ind Health. 1978;20:146-155 (in Japanese, English extended summary).

Alieva ZA, Sultanov MJ, Mirzoev TA. Reduced acuity of color perception resulting from exposure to styrene and tetrachloroethylene vapours.
Gig Trud Prof Zabol 1985;2:11-13 (in Russian, English Abstract).

Seppalainen,-A-M; Antti-Poika,-M. Time course of electrophysiological findings for patients with solvent poisoning. A descriptive study.
Scand-J-Work-Environ-Health. 1983 Feb; 9(1): 15-24
AB: The time course of electroencephalographic (EEG) and electroneuromyographic (ENMG) findings was studied among 87 patients (40 men and 47 women) with a diagnosis of chronic solvent intoxication after occupational exposure. Neurophysiological studies were initially performed around the time of diagnosis, and all the patients were reexamined three to nine years later. No control group was available for the follow-up. Upon diagnosis 67% of the patients had an abnormal EEG, the majority with diffuse slow-wave abnormalities. The reexamination showed improvement in the EEGs of 47%, but the percentage of patients with paroxysmal abnormalities had increased from the initial 6 to 17. The

percentage of patients with neuropathic findings was 62 for the first and 74 for the second ENMG. Upon reexamination fibrillations were seen in 46% (in 38% in the initial examination), and some loss of motor units was found for 61% (for 54% in the initial examination). Mild improvement in the neuropathic findings was noted for 60%, but the neuropathic findings of 25% showed slight deterioration during the follow-up period. The EEG findings showed a time course resembling that described after external head injuries. The ENMG findings resembled those described in hexa-carbon-induced or carbon disulfide-induced neuropathy. Controlled studies are recommended to examine the observed slight associations between electrophysiological findings and the type of chemical exposure.

Lindstrom,-K; Antti-Poika,-M; Tola,-S; Hyytiainen,-A. Psychological prognosis of diagnosed chronic organic solvent intoxication.

Neurobehav-Toxicol-Teratol. 1982 Sep-Oct; 4(5): 581-8

AB: The psychological prognosis of 86 patients (40 men and 46 women) with previously diagnosed chronic solvent intoxication due to trichloroethylene, perchloroethylene, or solvent mixtures was studied after a follow-up period (mean 5.9 years). The patients' mean age at the time of diagnosis was 38.6 years, and the mean duration of solvent exposure was 10.7 years. Tests for intelligence, short-term memory, and sensory and motor functions were applied. The group means of the patients' intellectual functions were increased after the follow-up period. At the group level, the scores on one sensorimotor task and tasks requiring manual dexterity were lower. Individual patients performed better, worse, or equally when the results of the initial examination and the reexamination were compared. The overall prognosis of psychological test results was better with a longer follow-up period and lower age. Patients who used drugs with neurological effects had a poorer overall psychological prognosis. The characteristic of solvent exposure were related only to the prognosis of some single tests for sensory and motor functions.

Antti-Poika,-M. Prognosis of symptoms in patients with diagnosed chronic organic solvent intoxication.

Int-Arch-Occup-Environ-Health. 1982; 51(1): 81-9

AB: The profile and prognosis of symptoms of 87 patients (mean age 38.6 years) in whom a chronic organic solvent intoxication due to tri- or perchloroethylene or mixtures of solvents had been diagnosed 3-9 years earlier were examined by means of an interview. Both at the time of diagnosis and upon reexamination, the most common symptoms were abnormal fatigue, memory disturbances and headache. Also dizziness, sleep disturbances, sensory symptoms in the extremities, mental depression, concentration difficulties, psychic irritability, emotional lability, tremor and nausea were present in over 60% of patients at the time of diagnosis. Upon reexamination, 52% of the intoxication patients with no other contributing neurological disease felt that their overall subjective condition was better than at the time of diagnosis, 21% felt that it was worse, and 27% reported no change. Most of the individual symptoms had more often changed for the better than for the worse; the differences were statistically significant with regard to abnormal fatigue, headache, dizziness, sleep disturbances, nausea, and emotional lability, whereas memory disturbances had changed in the opposite direction. Younger persons, who had had a longer follow-up period and without regular check-ups at the Institute of Occupational Health seemed to have better prognosis at the group level. Due to the great variation between the individuals, the prognosis was, however, impossible to predict in individual cases.

Antti-Poika,-M. Overall prognosis of patients with diagnosed chronic organic solvent intoxication.

Int-Arch-Occup-Environ-Health. 1982; 51(2): 127-38

AB: The prognosis of 87 patients (mean age 38.6 years) was examined who had been diagnosed 3-9 years earlier as having chronic organic solvent intoxication due to trichloroethylene, perchloroethylene, or a mixture of solvents. The methods comprised an interview, a clinical neurological examination, neurophysiological examinations (EEGs and electroneuromyographs), and a psychological examination. In order to assess the prognosis, the patient's condition at the time of diagnosis was evaluated from the hospital records, and the condition upon reexamination was compared to that at the time of diagnosis. At the time of diagnosis, 31 patients had objective clinical signs in the neurological examination, whereas the remaining 56 had only subjective symptoms and neurophysiological or psychological findings attributed to the slight solvent intoxication. Upon reexamination, 42 patients had clinical neurological signs. Based on

the clinical overall evaluation, the condition of 21 patients had deteriorated during the follow-up period, the condition of 23 had improved, and that of 43 had remained unchanged. The prognoses of the results of the separate examinations correlated poorly with each other. No statistically significant correlation was found between the overall prognosis and age, sex, the duration and the level of exposure, the termination of exposure after diagnosis, the presence of other diseases, or the use of alcohol.

Sinczuk-Walczak,-H. Stan ukladu nerwowego i czynnosci bioelektrycznej mozgu u pracownikow pralni chemicznych narazonych na czterochloroetylen. [Status of the nervous system and bioelectric activity of the brain in dry cleaning plant workers exposed to tetrachloroethylene].
Med-Pr. 1988; 39(2): 91-9 (Polish; English abstract)
AB: The studies covered 87 women and 44 men--workers of chemical laundries exposed to tetrachloroethylene (PER). About 50% of subjects were exposed, at the beginning of their employment, to trichloroethylene (TRI). In clinical examinations neuroses prevailed. The objective examination revealed disturbances of the vegetative system and symptoms of organic lesions of the central nervous system in form of encephalopathy. The EEG tests indicated paroxysmal lesions, usually with unaltered basic activities. Analysis of the rate of neurological lesions and EEG implied that the TRI-exposure at the beginning of employment did not have any significant effect on the clinical picture.

Sinczuk-Walczak,-H Analiza podstawowej czynnosci bioelektrycznej mozgu u pracownikow narazonych na przewlekle dzialanie niektorych rozpuszczalnikow organicznych. [Analysis of the basic bioelectric action of the brain in workers chronically exposed to certain organic solvents].
Med-Pr. 1994; 45(6): 473-8 (in Polish, English Abstract)
AB: The bioelectric action of the brain was analysed by means of EEC examination with an exclusive or almost exclusive alpha rythm in workers chronically exposed to tetrachloroethylene and in workers exposed to the mixture of solvents which contain aliphatic derivatives of benzene with nine or ten carbon atoms. The study covered 30 male workers of the similar age group, period of employment and work shifts. No symptoms of the nervous system damage were observed. There were either no significant differences in the frequency of alpha rythm according to EEC examination in workers exposed to organic solvents when compared with controls.

Polakowska,-B. Zaburzenia czynnosciowe ukladu nerwowego u osob narazonych zawodowo na mieszanine rozpuszczalnikow chloroorganicznych i chlorek winylu. [Functional disorders of the nervous system in those occupationally exposed to mixtures of chloro-organic solvents and vinyl chloride].
Med-Pr. 1990; 41(1): 39-43. (in Polish; English abstract)
AB: A group of 334 males, workers of the Nitrogen Plant in Wloclawek were examined: 172 subjects were employed in the department of solvents and vinyl chloride synthesis, 90 subjects in polyvinyl chloride processing department, and the rest of subjects formed a control group. Detailed neurological examinations made it possible to see that in the group occupationally exposed to solvents functional disorders of the nervous system of different intensities were observed (25.6%). The frequency of these changes in workers employed in particular departments characterized by different work conditions was compared, using statistical methods, with the results obtained in the control group. Functional disorders of the nervous system were found to be remarkably more frequent in the employees of the solvents production department where the greatest exposure to carbon tetrachloride and tetrachloroethylene were observed.

Muttray,-A; Randerath,-W; Ruhle,-K-H; Gajsar,-H; Gerhardt,-P; Greulich,-W; Konietzko,-J
Obstruktives Schlafapnoesyndrom durch eine berufliche Losungsmittelexposition.
[Obstructive sleep apnea syndrome caused by occupational exposure to solvents]
Dtsch-Med-Wochenschr. 1999 Mar 12; 124(10): 279-81 (in German, English Abstract).
AB: HISTORY AND ADMISSION FINDINGS: A 52-year-old man working in a chemical laboratory was referred with the possible diagnosis of toxic encephalopathy. For 17 years he had been exposed to high concentrations of

perchlorethylene and n-butanol vapours which every day had caused acute symptoms of organic solvent intoxication. Current complaints were autonomic nervous system symptoms, loss of concentration and memory, and fatigue in the second half of the day. The patient was obese but in good general condition. INVESTIGATIONS: Neuropsychiatric examination confirmed the reported loss of concentration and planning ability at work. The polysomnogram indicated an increased number of largely obstructive apnoea attacks. DIAGNOSIS, TREATMENT AND COURSE: As the patients had an obstructive type of sleep apnoea treatment consisted of positive pressure ventilation at night and weight reduction. The occupational exposure to organic solvents was the likely cause. CONCLUSIONS: As the symptoms of encephalopathy and sleep apnoea syndrome overlap, the latter should be considered before an encephalopathy is diagnosed. Because a rare cause of the sleep apnoea syndrome is prolonged and marked occupational exposure to organic solvents this should be asked about in taking the history. If indeed there has been occupational exposure, it should cease at once and be reported.

Gobba F, Cavalleri A
Color vision impairment in workers exposed to neurotoxic chemicals.
NeuroToxicology 2003;24:693-702.

Beliles RP. Concordance across species in the reproductive and developmental toxicity of tetrachloroethyene. Toxicol Ind Health 2002;18:91-106

Till et al. Assessment of visual functions following prenatal exposure to organic solvents. Neurotoxicology 2003;24:725-3.1

Till C, Westall CA, Rovet JF, Koren G. Effects of maternal occupational exposure to organic solvents on offspring visual functioning: a prospective controlled study. Teratology. 2001;64(3):134-41.

Gobba F
Occupational exposure to chemicals and sensory organs: a neglected research field
NeuroToxicology 2003;24:675-691.

Review by
William H. Merigan, Ph.D.

Review by William Merigan

General Impressions

The draft discussion paper, Neurotoxicity of Tetrachloroethylene, is a good summary and analysis of human and animal studies of effects of perc exposure on a wide range of neurotoxicity endpoints. It summarizes the major points of the relevant studies and then concludes, appropriately, that the nervous system is a target organ for both occupational and environmental perc exposure. I would prefer that in its final form the document provide a stronger critical analysis of the literature, particularly showing which conclusions can be drawn from each paper.

The first studies reviewed conducted experimental studies of human exposure to perc levels up to 100 ppm. Studies by Stewart and coworkers show dramatic neurotoxic effects (impaired balance, EEG changes, motor function) resulting from 100 ppm acute inhalation exposures of volunteers for several hours. More recent studies from Germany by Altmann and coworkers found consistent neurotoxicity (pattern VEP, contrast sensitivity, vigilence, eye hand coordination) at exposures of only 50 ppm. Although there are some inconsistencies between studies when similar measures were used, it seems clear from these studies that 50 to 100 ppm exposures, (which also cause eye and throat irritation) consistently produce a variety of neurobehavioral effects.

The document then examines a larger group of studies, carried out in several different countries, that examined chronically exposed populations including workers and some groups that lived near sites of perc use. A few of these studies report measurements that may also have been influenced by actute exposures, since testing was done shortly after acute exposure, for example, after several hours of work in a perc containing environment. Other studies in this section studied only chronic exposure by testing subjects at a clinic removed from the work site, or testing months after acute exposure had ceased. As might be expected, this group of studies was less consistent than the studies of experimentally exposed volunteers, with some studies failing to find particular effects that were consistently found in other studies. Nonetheless, as a group, these studies show that chronic exposure to perc, even in the absence of acute exposure immediately prior to testing, caused substantial alterations in neurobehavioral functions including reaction time, delayed choice responses, vigilance, etc. One study, (Schreiber) conducted visual contrast sensitivity testing, as well as color vision testing, in apartment dwellers exposed to perc by the proximity of their housing to a dry cleaning shop. Air monitoring suggested exposure peaks less that 1 ppm, but for a period of up to 6 years. Visual function for contrast sensitivity was significantly affected in these populations, especially among children, and these deficits appeared to be stable even months after the end of exposure. Two studies in Italy, by Cavalleri and Gobba, of the same group of perc workers, found decreased blue-yellow vision, and then further decreases in this measure of vision at a 2 year followup.

Finally, the document summarized numerous animal studies, conducted for the most part in rodents, with a wide range of concentrations and durations of exposure and a variety of endpoints. Although this data was not needed to establish that neurotoxic

effects at workplace exposure levels are likely in humans, these studies did show physiological, behavioral and neurochemical effects even during exposure to only moderate concentrations of perc. For example, brainstem auditory evoked potentials were disrupted at 50 ppm, DNA in frontal cortex at 60 ppm, and BuChE at only 37 ppm.

Response to charge questions:

Charge a. Strengths and limitations of human data, was it evaluated well?

Variety of exposure conditions leading to symptoms or findings – the reviewed literature shows neurotoxic effects due to perc exposure for a wide range of exposure conditions, ranging from experimental exposure of volunteers to 50 or 100 ppm to chronic occupational exposure to apparently lower levels of perc, and finally to chronic environmental exposure to yet lower levels. It seems remarkable to me, although it was solidly established by the reviewed studies, that such diverse exposures all resulted in neurotoxic endpoints, many quite similar. This consistency of finding impaired neural function across such diverse conditions, together with the plethora of subjective symptoms at these similar exposure levels, suggests to me that the perc exposure in these studies caused consistent effects and that exposure levels would have to be severely decreased to avoid producing neurotoxic effects.

Variety of measures - the range of neuronal functions affected by perc in these studies was also extraordinary, including impaired equilibrium on the Romberg test, several indices of reduced visual performance, disrupted vigilance, choice reaction time, pattern memory, etc. A skeptic might complain that many largely unrelated measures were obtained in each study, increasing the likelihood that some effect would be found. On the other hand, each of the measured functions was also compared to observations in a control group, and in many cases the tests were conducted in single-blind fashion, making the findings of impairment convincing. It is possible that focus on the most sensitive measures of perc neural effects might show neurotoxic effects at much lower exposure levels.

Clean experimental design in several studies – in many of the reported studies every effort seemed to have been made to rule out experimenter bias. For example in the studies of Cavalleri and Gobba, experimental and control subjects were tested at the same time, and with the same lighting conditions, by investigators who did not know which group they were in. Such precautions increase a reviewer's confidence that the findings represent perc effects. Correlation between the index of neurotoxicity and exposure indices in other studies also support the view that the reported effects were due to perc.

No convergence on mechanism – I found little hint in the many reported effects that there was a narrow mechanism of effect, and that the tests chosen were targeted toward that mechanism. Although several of the reported measures involved visual function, I could see no reason for believing that perc effects were more pronounced on the retina than on

other portions of the nervous system. In fact, many findings (e.g. digit reproduction, pattern recognition) suggested a likely effect on high-level brain functions, and it remains possible that color vision and contrast sensitivity effects could also involve impaired brain function. This is not a criticism of the reviewed literature. I am not sure which measures I would choose as the most sensitive measures or those related to a likely mechanism of tocxicity. (parenthetically, Diana Echevarria reported during the meeting that her extensive findings, some published, some in preparation, suggested that the toxicity of perc was dramatic for visually presented tests, but not for tests that avoided vision. I find this a surprising result and would like to see the papers)

Inability to determine vulnerable populations – although particular populations (children, elderly) can be more vulnerable to toxicant exposure, the reviewed studies of perc did not permit the assessment of special vulnerabilities. The issue was discussed in the Schreiber paper, but the study population in this paper was not appropriate for determining if there is special vulnerability.

Overview – the authors of the discussion paper did an excellent job of reviewing the many forms of perc neurotoxicity, in the many populations affected. My concern, after examining this literature, is that perc effects (some of which appear to be irreversible) may extend to exposures well below those studied to date. ***The points outlined above***, that perc exposure results in serious neurotoxicity, that this shows up in numerous measures and across different types of exposed groups, that there is presently no convergence on mechanism, and that possible vulnerable populations have not been examined, ***suggest that perc neurotoxicity could be much worse than we currently understand.***

Charge b. ***contrast sensitivity and other visual effects*** **-** In the studies reviewed in the discussion document were three reports of perc effects on color confusion or contrast sensitivity.

Cavalleri and Gobba found increased color confusion index (CCI) in perc workers, and then found continued decline in CCI in the same workers two years later. These findings are especially powerful because the reports make clear that the measures were obtained under conditions that make them very solid (testers were blind, visual conditions were exactly the same for both groups). Also they are possibly irreversible.

Schreiber et. al. found substantial contrast sensitivity reductions in apartment dwellers and day care workers exposed to perc. Again investigators were unaware of which subjects were experimental and which control. This study also measured color confusion index in the same exposed populations (using the same procedures as used in the studies above), and found that CCI was not significantly decreased in either group. This comparison suggests that the contrast sensitivity measure may have been more sensitive than the color confusion index (although such a comparison is probably unwarranted, since choice of test conditions (size of test stimulus, use of forced choice versus detection

test method, luminance of test stimuli, etc.) might greatly affect sensitivity. If investigators had a clear idea of the mechanism of the neurotoxicity they could probably design the tests to be more sensitive, and possibly more selective.

Altmann also measured contrast sensitivity in volunteers exposed to 10 or 50 ppm perc. The discussion paper reports that CS was decreased more by 50 than by 10 ppm perc, but says no statistical tests were run, making it unclear if CS was reliably decreased. My reading of this report did not inspire confidence that CS was actually affected by perc. I recall that four of six measures showed lower sensitivity, two showed higher and one was unchanged.
The only other simple visual tests conducted in the other papers were acuity, in three papers, and flicker fusion in the study of Lauwerys, and these measures were unaffected by perc.

> In addition, a large number of complex, visually mediated tasks were examined in these studies, but there were no obvious common features between those performances that were affected (delayed reading, pattern recognition, perceptual speed, choice reaction time, vigilance, visual memory, etc.) and those that were not significantly affected (eye hand coordination, sustained attention, simple reaction time, digit symbol test, etc.).

In addition, visual evoked potentials were studied in two studies reported by Altmann. In one case, VEP waveforms were distorted, but in the second case the latency of a few of the measured peaks was increased. Hard to tell what the mechanism of this change is.

Overview – there is too much variety in the visual measures that have been used for me to determine a common thread. Many of the reported effects clearly involve impaired neural function within the brain (e.g. impaired Romberg test), so it must be asked if all perc neurotoxic effects could, in principle, be due to generalized brain dysfunction. Color discriminations and contrast sensitivity are certainly dependent on thalamic and cortical brain function, as well as on retina, and if they indeed show greater sensitivity to perc exposure than other measures, this could simply reflect their excellence as indices of nervous system function.

Charge c. Biological and clinical significance of endpoints in Table 1.
Virtually all are of serious concern as neurotoxic effects in their own right, and could also lead to serious problems due to inattention to dangers, impaired self-rescue, etc.

Charge d. Weight assigned to residential studies?
Great weight should be assigned to these studies, given that the effects seem clear and are not weakened by poor experimental design, they do not depend on acute exposure to perc, and some may be irreversible.

Charge e. Susceptible populations

There is no compelling evidence that particular populations are at greater risk for perc exposure, but this is inevitable given that different populations were not examined systematically. The contrast sensitivity study mentioned effects in children and elders, but I found nothing indicating that they showed particularly severe effects. (however, the discussion document suggested that contrast sensitivity changes were more dramatic in children and elders. Questins during the public review suggested that this information was gathered by personal contact with the investigators. This should be pointed out in the EPA document)

Charge f. Do neurologic effects of other solvents support perc effects?
Yes, the effects of other solvents are also diffuse, but with frequent observations of color vision effects, dizziness, etc. One particularly relevant paper on CS in electronics workers by Broadbent, Hudnell, Boyes et al? was discussed at the review session. David Bottimore received this paper.

Charge g. Overview, do these studies show perc neurotoxicity?
Yes, the cumulative weight of all the studies indicate that perc represents a serious health risk, even at levels at which workers and bystanders are routinely exposed.

Charge h. Published papers not included?
The recently published paper by Dick et. al. 2004, as well as other studies showing color vision effects of solvent exposure, should be included in all further analyses of perc effects.

Specific observations about the discussion document
Page 3, line 13: is this the odor of the perc, or did subjects lose their ability to make olfactory discriminations.

Page 6, line 14: is the Altmann study still of interest in determining the acute neurotoxicity of perc?

Page 7, line 24: given the importance of contrast sensitivyt for perc, this section should be expanded.

Page 7, line 34: "degree of improvement"? under what conditions?

Page 8, line 30-32: Should non-significant effects be discussed as suggestive of subtle effects?

Page 16, line 13-15: These two conclusions include all possibilities (either retinal or post retinal), and therefore have no content. I agree that it is probably impossible to decide on the basis of psychophysical tests.

Page 24, line 2-3: I do not think that a lack of significant acuity effect can be used to rule out optical (e.g. accommodation) effects**.**

Page 24, line 24ff: This discussion describes effects on children that I did not find in the paper (can someone point me to the right section). Also, the discussion of small and large objects is not consistent with current theory.

Page 26, line 21-22: I am also surprised, but I don't know if this statement suggests more (e.g.that the CS effects are not correct)?

Page 27, line 25: I like the effort to extract common features of the disrupted versus spared functions, but I do not think a strong case can be made for commonality of "visuospatial" performance.

Appendix A- Agenda

United States
Environmental Protection Agency
Office of Research and Development

Peer Consultation of *Neurotoxicity of Tetrachloroethylene (Perchloroethylene) Discussion Paper*

Crystal Clubroom
Marriott Crystal City Hotel
1999 Jefferson Davis Highway
Arlington, VA 22202

Agenda

WEDNESDAY, FEBRUARY 25, 2004

8:15AM	**Registration Begins**
8:30AM	**Welcome, Introductions, and Goals of Meeting** David Bottimore, Versar, Inc.
8:45AM	**Welcome** David Bussard, Director of the Washington Division, National Center for Environmental Assessment (NCEA), U.S. EPA
8:50AM	**Chair's Introduction** Kent Anger, Workshop Chair
9:00AM	**Roundtable Overview of Reviewer Comments**
9:30AM	**Discussion Session (w/ break) -** Charge Questions a, b, and c
11:45AM	Lunch
1:00PM	**Discussion Session (w/ break) -** Charge Questions d, e, f, and g
3:30PM	**Recap of Comments and Recommendations**
4:00PM	**Observer Comment Period**

4:30PM **Closing Remarks and Adjourn**

Appendix B – List of Observers

Peer Consultation of
Neurotoxicity of Tetrachloroethylene (Perchloroethylene) Discussion Paper

February 25, 2004

OBSERVERS	
Name	**Organization**
Stanley Barone, Jr.	U. S. EPA - ORD/NCEA
Nancy Beck	OMB
William K. Boyes	U.S. EPA - ORD/NHEERL
Rebecca Brown	ASPH/EPA
David Bussard	U.S. EPA - ORD/NCEA
George Cruzan	ToxWorks
Paul H. Dugard	Halogenated Solvents Industry Alliance, Inc.
Steve Gibb	Inside EPA's Risk Policy Report
H. Kenneth Hudnell	U.S. EPA
William E. Luttrell	Navy Environmental Health Center
Robert McGaughy	U.S. EPA - ORD/NCEA
W. Caffey Norman	Patton Boggs LLP
Mary Beth Polley	Pesticide and Toxic Chemical News
Peter W. Preuss	U.S. EPA - ORD/NCEA
Steve Risotto	Halogenated Solvents Industry Alliance, Inc.
Judith S. Schreiber	New York Office of the Attorney General
Cheryl Siegel Scott	U.S. EPA - ORD/NCEA
Bob Sonawane	U.S. EPA - ORD/NCEA
Chad Thompson	AAAS/EPA