

## **APPENDIX D**

# **Neurological Effects of Trichloroethylene**

*This document is a draft for review purposes only and does not constitute Agency policy.*

10/20/09

D-i

DRAFT—DO NOT CITE OR QUOTE

## CONTENTS—Appendix D: Neurological Effects of Trichloroethylene

LIST OF TABLES .....	D-iii
APPENDIX D. NEUROLOGICAL EFFECTS OF TRICHLOROETHYLENE .....	D-1
D.1. HUMAN STUDIES ON THE NEUROLOGICAL EFFECTS OF TRICHLOROETHYLENE (TCE) .....	D-1
D.1.1. Changes in Nerve Conduction .....	D-1
D.1.1.1. Blink Reflex and Masseter Reflex Studies—Trigeminal Nerve .....	D-1
D.1.1.2. Trigeminal Somatosensory Evoked Potential (TSEP) Studies—Trigeminal Nerve .....	D-6
D.1.1.3. Nerve Conduction Velocity Studies .....	D-9
D.1.2. Auditory Effects .....	D-10
D.1.3. Vestibular Effects .....	D-13
D.1.4. Visual Effects .....	D-16
D.1.5. Cognition .....	D-18
D.1.6. Psychomotor Effects .....	D-22
D.1.6.1. Reaction Time .....	D-22
D.1.6.2. Muscular Dyscoordination .....	D-26
D.1.7. Summary Tables .....	D-28
D.2. CENTRAL NERVOUS TOXICITY IN ANIMAL STUDIES FOLLOWING TRICHLOROETHYLENE (TCE) EXPOSURE .....	D-90
D.2.1. Alterations in Nerve Conduction .....	D-90
D.2.2. Auditory Effects .....	D-92
D.2.2.1. Inhalation .....	D-92
D.2.2.2. Oral and Injection Studies .....	D-95
D.2.3. Vestibular System Studies .....	D-95
D.2.4. Visual Effects .....	D-96
D.2.5. Cognitive Function .....	D-98
D.2.6. Psychomotor Effects .....	D-99
D.2.6.1. Loss of Righting Reflex .....	D-99
D.2.6.2. Functional Observational Battery (FOB) and Locomotor Activity Studies .....	D-100
D.2.6.3. Locomotor Activity .....	D-102
D.2.7. Sleep and Mood Disorders .....	D-104
D.2.7.1. Effects on Mood: Laboratory Animal Findings .....	D-104
D.2.7.2. Sleep Disturbances .....	D-104
D.2.8. Mechanistic Studies .....	D-104
D.2.8.1. Dopaminergic (DA) Neurons .....	D-104
D.2.8.2. Gamma-Amino Butyric Acid (GABA) and Glutamatergic Neurons .....	D-105
D.2.8.3. Demyelination Following Trichloroethylene (TCE) Exposure .....	D-107
D.2.9. Summary Tables .....	D-109
D.3. REFERENCES .....	D-122

*This document is a draft for review purposes only and does not constitute Agency policy.*

**LIST OF TABLES**

D-1. Epidemiological studies: Neurological effects of trichloroethylene..... D-30

D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents ..... D-68

D-3. Literature review of studies of TCE and domains assessed with neurobehavioral/neurological methods..... D-88

D-4. Summary of mammalian *in vivo* trigeminal nerve studies..... D-110

D-5. Summary of mammalian *in vivo* ototoxicity studies..... D-111

D-6. Summary of mammalian sensory studies—vestibular and visual systems..... D-113

D-7. Summary of mammalian cognition studies..... D-114

D-8. Summary of mammalian psychomotor function, locomotor activity, and reaction time studies ..... D-115

D-9. Summary of mammalian *in vivo* dopamine neuronal studies ..... D-117

D-10. Summary of neurochemical effects with TCE exposure ..... D-118

D-11. Summary of *in vitro* ion channel effects with TCE exposure..... D-120

D-12. Summary of mammalian *in vivo* developmental neurotoxicity studies—oral exposures..... D-121

*This document is a draft for review purposes only and does not constitute Agency policy.*

## APPENDIX D. NEUROLOGICAL EFFECTS OF TRICHLOROETHYLENE

### D.1. HUMAN STUDIES ON THE NEUROLOGICAL EFFECTS OF TRICHLOROETHYLENE (TCE)

There is an extensive body of evidence in the literature on the neurological effects caused by exposure to trichloroethylene (TCE) in humans. The primary functional domains that have been studied and reported are trigeminal nerve function and nerve conductivity (latency), psychomotor effects, including reaction times (simple and choice), visual and auditory effects, cognition, memory, and subjective neurological symptoms, such as headache and dizziness. This section discusses the primary studies presented for each of these effects. Summary tables for all the human TCE studies are at the end of this section.

#### D.1.1. Changes in Nerve Conduction

There is strong evidence in the literature that exposure to TCE results in impairment of trigeminal nerve function in humans exposed occupationally, by inhalation, or environmentally, by ingestion. Functional measures such as the blink reflex and masseter reflex tests were used to determine if physiological functions mediated by the trigeminal nerve were significantly impacted. Additionally, trigeminal somatosensory evoked potentials were also measured in some studies to ascertain if nerve activity was directly affected by TCE exposure.

##### D.1.1.1. *Blink Reflex and Masseter Reflex Studies—Trigeminal Nerve*

Barret et al. (1984) conducted a study on 188 workers exposed to TCE occupationally from small and large factories in France (type of factories not disclosed). The average age of the workers was 41 (standard deviation [SD] not provided, but authors noted 14% <30 years and 25% >50 years) and the average exposure duration was 7 hours/day for 7 years. The 188 workers were divided into high and low exposure groups for both TCE exposure measured using detector tubes and trichloroacetic acid (TCA) levels measured in urine. There was no unexposed control population, but responses in the high-exposure group were compared response in the low-exposure group. TCE exposure groups were divided into a low exposure group (<150 ppm;  $n = 134$ ) and a high exposure group (>150 ppm;  $n = 54$ ). The same workers ( $n = 188$ ) were also grouped by TCA urine measurements such that a high exposure was  $\geq 100$  mg TCA/g creatinine. Personal factors including age, tobacco use and alcohol intake were also analyzed. No mention was made regarding whether or not the examiners were blind to the subjects' exposure status. Complete physical examination including testing visual performance (acuity and color perception), evoked trigeminal potential latencies and audiometry, facial

*This document is a draft for review purposes only and does not constitute Agency policy.*

sensitivity, reflexes, and motoricity of the masseter muscles. Chi squared analysis was used to examine distribution of the different groups for comparing high and low exposed workers followed by one way analysis of variance. Overall, 22 out of 188 workers (11.7%) experienced trigeminal nerve impairment ( $p < 0.01$ ) as measured by facial sensitivity, reflexes (e.g., jaw, corneal, blink) and movement of the masseter muscles. When grouped by TCE exposure, 12 out of 54 workers (22.2%) in the high exposure group ( $\geq 150$  ppm) and 10 out of 134 workers (7.4%) in the low exposure group had impaired trigeminal nerve mediated responses. When grouped by the presence of TCA in the urine, 41 workers were now in the high TCA group and 10 out of 41 workers (24.4%) experienced trigeminal nerve impairment in comparison to the 12 out of 147 (8.2%) in the low TCA ( $< 100$  mg TCA/g creatinine) group. Statistically significant results were also presented for the following symptoms based on TCE and TCA levels: trigeminal nerve impairment ( $p < 0.01$ ), asthenia ( $p < 0.01$ ), optic nerve impairment ( $p < 0.001$ ), and dizziness ( $0.05 < p < 0.06$ ). Statistically significant results were also presented for the following symptoms based on TCA levels: Trigeminal nerve impairment ( $p < 0.01$ ), asthenia ( $p < 0.01$ ), optic nerve impairment ( $p < 0.001$ ), headache ( $p < 0.05$ ), and dizziness ( $0.05 < p < 0.06$ ). Symptoms for which there is a synergistic toxic role for TCE and alcohol ( $p < 0.05$ ) were liver impairment and degreaser flush. This study presents a good statistically significant dose-response relationship between TCE/TCA exposure and trigeminal nerve impairment. TCE concentrations are not available for individual subjects, but exposure assessment was inferred based on occupational standards at the time of the study.

Feldman et al. (1988) conducted an environmental study on 21 Woburn, Massachusetts residents with alleged chronic exposure to TCE in drinking water, resulting from an environmental spill by a local industry. These were from 8 families whose drinking water wells were found to be contaminated with TCE and other solvents. The subjects were self selected, having been referred for clinical evaluation due to suspected neurotoxicity, and were involved in litigation. The control group was 27 unexposed residents from a nearby community with TCE concentrations in drinking water below state standards. TCE in residential well water was measured over a prior 2 year period (1979–1981); the maximum reported concentration for the study population was 267 ppb. The residents' water supply came from two different TCE-contaminated wells that had an average measured concentration of 256 ppb (labeled "Well G" based on 6 samples) and 111 ppb (labeled "Well H;" based on 4 samples). The residents' exposure ranged from 1–12 years and was dependent on the length of residence and the age of the subject. There were other solvents found to be present in the well water, and TCE data were not available for the entire exposure period. TCE concentrations for the control population were less than the maximum contaminant level (MCL) (5 ppb). The BR was used to measure the neurotoxic effects of TCE. The BR was measured using an electrode to stimulate the

*This document is a draft for review purposes only and does not constitute Agency policy.*

supraorbital nerve (above the eyelid) with a shock (0.05 ms in duration) resulting in a response and the response was measured using a recording electrode over the orbicularis oculi muscle (the muscle responsible for closing the eyelid and innervated by the trigeminal nerve). The BR generated an R1 and an R2 component from each individual. BRs were recorded and the supraorbital nerve was stimulated with single electrical shocks of increasing intensity until nearly stable R1 and R2 ipsilateral and R2 contralateral responses were obtained. The student's t-test was used for testing the difference between the group means for the blink reflex component latencies. Because of the variability of R2 responses, this study focused primarily on the R1 response latencies. Highly significant differences in the conduction latency means of the BR components for the TCE exposed population versus control population were observed when comparing means for the right and left side R1 to the controls. The mean R1 BR component latency for the exposed group was 11.35 ms, SD = 0.74 ms, 95% confidence interval (CI): 11.03–11.66. The mean for the controls was 10.21 ms, SD = 0.78 ms, 95% CI: 9.92–10.51; ( $p < 0.001$ ). The study was well conducted with consistency of methods, and statistically significant findings for trigeminal nerve function impairment resulting from environmental exposures to TCE. However, the presence of other solvents in the well water, self selection of subjects involved in litigation, and incomplete characterization of exposure present problems in drawing a clear conclusion of TCE causality or dose-response relationship.

Kilburn and Warshaw (1993) conducted an environmental study on 544 Arizona residents exposed to TCE in well-water. TCE concentrations were from 6 to 500 ppb and exposure ranged from 1 to 25 years. Subjects were recruited and categorized in 3 groups. Exposed group 1 consisted of 196 family members with cancer or birth defects. Exposed group 2 consisted of 178 individuals from families without cancer or birth defects; and exposed group 3 included 170 parents whose children had birth defects and rheumatic disorders. Well-water was measured from 1957 to 1981 by several governmental agencies and average annual TCE exposures were calculated and then multiplied by each individual's years of residence for 170 subjects. A referent group of histology technicians ( $n = 113$ ) was used as a comparison for the BR test. For this test, recording electrodes were placed over the orbicularis oculi muscles (upper and lower) and the BR was elicited by gently tapping the glabella (located on the mid-frontal bone at the space between the eyebrows and above the nose). A two-sided Student's t-test and linear regression were used for statistical analysis. Significant increases in the R1 component of the BR response was observed in the exposed population as compared to the referent group. The R1 component measured from the right eye appeared within 10.9 ms in TCE-exposed subjects whereas in referents, this component appeared 10.2 ms after the stimulus was elicited indicating a significant delay ( $p < 0.008$ ) in the reflex response. Similarly, delays in the latency of appearance for the R1 component were also noted for the left eye but the effect

*This document is a draft for review purposes only and does not constitute Agency policy.*

was not statistically significant ( $p = 0.0754$ ). This study shows statistically significant differences in trigeminal nerve function between subjects environmentally exposed and nonexposed to TCE. This is an ecological study with TCE exposure inferred to subjects by residence in a geographic area. Estimates of TCE concentrations in drinking water to individual subjects are lacking. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Kilburn (2002a) studied 236 residents (age range: 18–83 years old) lived nearby manufacturing plants (e.g., microchip plants) in Phoenix, AZ. Analysis of the groundwater in the residential area revealed contamination with many volatile organic compounds including TCE. Concentrations of TCE in the well water ranged from 0.2 ppb to more than 10,000 ppb and the exposure duration varied between 2 to 37 years. Additional associated solvents included dichloroethane (DCE), perchloroethylene, and vinyl chloride. A group-match design was used to compare the 236 TCE-exposed residents to 161 unexposed regional referents and 67 referents in NE Phoenix in the BR test. The BR response was recorded from surface electrodes placed over the location of the orbicularis oculi muscles. The reflex response was elicited by gently tapping the left and right supraorbital notches with a small hammer. The R1 component of the BR response was measured for both the left and right eye. Statistically significant increases in latency time for the R1 component was observed for residents exposed to TCE in comparison to the control groups. In unexposed individuals, the R1 component occurred within 13.4 ms from the right eye and 13.5 ms from the left eye. In comparison, the residents near the manufacturing plant had latency times of 14.2 ms ( $p < 0.0001$ ) for the right eye and 13.9 ms ( $p < 0.008$ ) for the left eye. This study shows statistically significant differences between environmentally exposed and unexposed populations for trigeminal nerve function, as a result of exposures to TCE. This is an ecological study with TCE exposure potential to subjects inferred by residence in a geographic area. Estimates of TCE concentrations in drinking water to individuals are lacking. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Feldman et al. (1992) evaluated the BR reflex in 18 subjects occupationally exposed to neurotoxic chemicals (e.g., degreasers, mechanics, and pesticide sprayers among many others). Eight of the subjects were either extensively ( $n = 4$ ) or occupationally ( $n = 4$ ) exposed to TCE. The remaining subjects ( $n = 10$ ) were exposed to other neurotoxic chemicals, but not TCE. Quantitative exposure concentration data were not reported in the study, but TCE exposure was characterized as either “extensive” or “occupational.” Subjects in the “extensive” exposure group were chronically exposed ( $\geq 1$  year) to TCE at least 5 days a week and for at greater than 50% of the workday ( $n = 3$ ) or experienced a direct, acute exposure to TCE for greater than 15 minutes ( $n = 1$ ). Subjects in the “occupational” group were chronically exposed ( $\geq 1$  year) to

*This document is a draft for review purposes only and does not constitute Agency policy.*

TCE for 1–3 days/week and for greater than 50% of the workday. The BR responses from the TCE-exposed subjects were compared to a control group consisting of 30 nonexposed subjects with no noted neurological disorders. BR responses were measured using surface electrodes over the lower lateral portion of the orbicularis oculi muscle. Electrical shocks with durations of 0.05 ms were applied to the supraorbital nerve to generate the R1 and R2 responses. All of the subjects that were extensively exposed to TCE had significantly increased latency times in the appearance of the R1 component (no *p*-value listed) and for 3 subjects this increased latency time persisted for at least 1 month and up to 20 years postexposure. However, none of the subjects occupationally exposed to TCE had changes in the BR response in comparison to the control group. In comparing the remaining neurotoxicant exposed subjects to the TCE-exposed individuals, the sensitivity, or the ability of a positive blink reflex test to identify correctly those who had TCE exposure was 50%. However, in workers with no exposure to TCE, 90% demonstrated a normal R1 latency.

Mixed results were obtained in a study by Ruitjen et al. (1991) on 31 male printing workers exposed to TCE. The mean age was 44; mean exposure duration was 16 years and had at least 6 years of TCE exposure. The control group consisted of 28 workers with a mean age 45 years. Workers in the control group were employed at least 6 years in print factories (similar to TCE-exposed), had no exposure to TCE, but were exposed to “turpentine-like organic solvents.” TCE exposure potential was inferred from historical monitoring of TCE at the plant using gas detection tubes. These data indicated TCE concentrations in the 1960s of around 80 ppm, mean concentration of 70 ppm in the next decade, with measurements from 1976 and 1981 showing a mean concentration of 35 ppm. The most recent estimate of TCE concentrations in the factory was 17 ppm (stable for 3 years) at the time of the report. The authors calculated that mean cumulative TCE exposure would be 704 ppm × years worked in factory. The masseter and blink reflexes were measured to evaluate trigeminal nerve function in TCE-exposed and control workers. For measurement of the masseter reflex, surface electrodes were attached over the right masseter muscle (over the cheek area). A gentle tap on a roller placed under the subject’s chin was used to elicit the masseter reflex. For measurement of the blink reflex, surface electrodes were placed on the muscle near the upper eyelid. Electrical stimulation of the right supraorbital nerve was used to generate the blink reflex. There was a significant increase in the latency of the masseter reflex to appear for the TCE-exposed workers (*p* < 0.05). However, there was no significant change in the blink reflex measure between TCE-exposed workers and control. Although no change in the blink reflex measures were observed between the two groups, it should be noted that the control group was exposed to other volatile organic solvents (not specified) and this volatile organic compound exposure could be a possible confounder for determination of TCE-induced effects.

*This document is a draft for review purposes only and does not constitute Agency policy.*



There are two studies that reported no effect of TCE exposure on trigeminal nerve function (El-Ghawabi et al., 1973; Rasmussen et al., 1993c). El-Ghawabi et al. (1973) conducted a study on 30 money printing shop workers occupationally exposed to TCE. Metabolites of total trichloroacetic acid and trichloroethanol were found to be proportional to TCE concentrations up to 100 ppm (550 mg/m<sup>3</sup>). Controls were 20 age- and socio-economic status (SES)-matched nonexposed males and 10 control workers not exposed to TCE. Trigeminal nerve involvement was not detected, but the authors failed to provide details as to how this assessment was made. It is mentioned that each subject was clinically evaluated and trigeminal nerve involvement may have been assessed through a clinical evaluation. As a result, the conclusions of this study are tempered since the authors did not provide details as to how trigeminal nerve function was evaluated in this study.

Rasmussen et al. (1993c) conducted an historical cohort study on 99 metal degreasers. Subjects were selected from a population of 240 workers from 72 factories in Denmark. The participants were divided into three groups based on solvent exposure durations where low exposure was up to 0.5 years, medium was 2.1 years and high was 11.0 years (mean exposure duration). Most of the workers (70 out of 99) were primarily exposed to TCE with an average exposure duration of 7.1 years for 35 hours/week. TCA and trichloroethanol (TCOH) levels were measured in the urine samples provided by the workers and mean TCA levels in the high group was 7.7 mg/L and was as high as 26.1 mg/L. Experimental details of trigeminal nerve evaluation were not provided by the authors. It was reported that 1 out of 21 people (5%) in the low exposure, 2 out of 37 (5%) in the medium exposure and 4 out of 41 (10%) in the high exposure group experienced abnormalities in trigeminal nerve sensory function. No linear association was seen on trigeminal nerve function (Mantel-Haenzel test for linear association,  $p = 0.42$ ). However, the trigeminal nerve function findings were not compared to a control (no TCE exposure) group and it should be noted that some of the workers (29 out of 99) were not exposed to TCE.

#### **D.1.1.2. Trigeminal Somatosensory Evoked Potential (TSEP) Studies—Trigeminal Nerve**

In a preliminary study, Barret et al. (1982) measured trigeminal sensory evoked potentials (TSEPs) in eleven workers that were chronically exposed to TCE. Nine of these workers were suffering effects from TCE intoxication (changes in facial sensitivity and clinical changes in trigeminal nerve reflexes), and two were TCE-exposed without exhibiting any clinical manifestations from exposure. A control group of 20 nonexposed subjects of varying ages were used to establish the normal response curve for the trigeminal nerve function. In order to generate a TSEP, a surface electrode was placed over the lip and a voltage of 0.05 ms in duration was applied. The area was stimulated 500 times at a rate of two times per second. TSEPs were

*This document is a draft for review purposes only and does not constitute Agency policy.*

recorded from a subcutaneous electrode placed between the international CZ point (central midline portion of the head) and the ear. In eight of the eleven workers, an increased voltage ranging from a 25 to a 45 volt increase was needed to generate a normal TSEP. Two of the 11 workers had an increased latency of appearance for the TSEP and three workers had increases in TSEP amplitudes. The preliminary findings indicate that TCE exposure results in abnormalities in trigeminal nerve function. However, the study does not provide any exposure data and lacks information with regards to the statistical treatment of the observations.

Barret et al. (1987) conducted a study on 104 degreaser machine operators in France (average age = 41.6 years; range = 18–62 years) who were highly exposed to TCE with an average exposure of 7 hours/day for 8.23 years. Although TCE exposure concentrations were not available, urinary concentrations of TCOH and TCA were measured for each worker. A control group consisting of 52 subjects without any previous solvent exposure and neurological deficits was included in the study. Trigeminal nerve symptoms and TSEPs were collected for each worker. Trigeminal nerve symptoms were clinically assessed by examining facial sensitivity and reflexes dependent on this nerve such as the jaw and blink reflex. TSEPs were elicited by electrical stimulation (70–75 V for 0.05 ms) of the nerve using an electrode on the lip commissure. Eighteen out of 104 TCE-exposed machine operators (17.3%) had trigeminal nerve symptoms. The subjects that experienced trigeminal nerve symptoms were significantly older (47.8 years vs. 40.5;  $p < 0.001$ ). Both groups had a similar duration of exposure with a mean of 9.2 years in the sensitive group and 7.8 years in the nonsensitive group. Urinary concentrations of TCOH and TCA were also statistically similar although the levels were slightly higher in the sensitive group (245 mg/g creatinine vs. 162 mg/g creatinine for TCOH; 131 mg/g creatinine vs. 93 mg/g creatinine for TCA). However, in the same group, 40 out of 104 subjects (38.4%) had an abnormal TSEP. Abnormal TSEPs were characterized as potentials that exhibited changes in latency and/or amplitude that were at least 2.5 times the standard deviation of the normal TSEPs obtained from the control group. Individuals with abnormal TSEP were significantly older (45 years vs. 40.1 years;  $p < 0.05$ ) and were exposed to TCE longer (9.9 years vs. 5.6 years;  $p < 0.01$ ). Urinary concentrations TCOH and TCA were similar between the groups with sensitive individuals having average metabolite levels of 195 mg TCOH/g creatinine and 98.3 mg TCA/g creatinine in comparison to 170 mg TCOH/g creatinine and 96 mg TCA/g creatinine in nonsensitive individuals. When a comparison was made between workers that had normal TSEP and no trigeminal symptoms and workers that had an abnormal TSEP and experienced trigeminal symptoms, it was found that in the sensitive individuals (abnormal TSEP and trigeminal symptoms) there was a significant increase in age (48.5 vs. 39.5 years old,  $p < 0.01$ ), duration of exposure (11 vs. 7.5 years,  $p < 0.05$ ) and an increase in urinary TCA (313 vs. 181 mg TCA/g creatinine). No significant changes were noted in urinary TCOH, but the

*This document is a draft for review purposes only and does not constitute Agency policy.*

levels were slightly higher in sensitive individuals (167 vs. 109 mg TCOH/g creatinine). Overall, it was concluded that abnormal TSEPs were recorded in workers who were exposed to TCE for a longer period (average duration 9.9 years). This appears to be a well designed study with statistically significant results reported for abnormal trigeminal nerve response in TCE exposed workers. Exposure assessment to TCE is by exposure duration and mean urinary TCOH and TCA concentrations. TCE concentrations to exposed subjects as measured by atmospheric or personal monitoring are lacking.

Mhiri et al. (2004) measured TSEPs from 23 phosphate industry workers exposed to TCE for 6 hours/day for at least two years while cleaning tanks. Exposure assessment was based on measurement of urinary metabolites of TCE, which were performed 3 times/worker, and air measurements. Blood tests and hepatic enzymes were also collected. The mean exposure duration was  $12.4 \pm 8.3$  years (exposure duration range = 2–27 years). Although TCE exposures were not provided, mean urinary concentrations of TCOH, TCA, and total trichlorides were  $79.3 \pm 42$ ,  $32.6 \pm 22$ , and  $111.9 \pm 55$  mg/g urinary creatinine, respectively. The control group consisted of 23 unexposed workers who worked in the same factory without being exposed to any solvents. TSEPs were generated from a square wave pulses (0.1 ms in duration) delivered through a surface electrode that was placed 1 cm under the corner of the mouth. The responses to the stimuli (TSEPs) were recorded from another surface electrode that was placed over the contralateral parietal area of the brain. The measured TSEP was divided into several components and labeled according to whether it was (1) a positive (P) or negative (N) potential and (2) the placement of the potential in reference to the entire TSEP (e.g., P1 is the first positive potential in the TSEP). TSEPs generated from the phosphate workers that were  $\pm 2.5$  times the standard deviation from the TSEPs obtained from the control group were considered abnormal. Abnormal TSEP were observed in 6 workers with clinical evidence of trigeminal involvement and in 9 asymptomatic workers. Significant increases in latency were noted for all TSEP potentials (N1, P1, N2, P2, N3,  $p < 0.01$ ) measured from the phosphate workers. Additionally, significant decreases in the P1 ( $p < 0.02$ ) and N2 ( $p < 0.05$ ) amplitudes were observed. A significant positive correlation was demonstrated between duration of exposure and the N2 latency ( $p < 0.01$ ) and P2 latency ( $p < 0.02$ ). Only one subject had urinary TCE metabolite levels over tolerated limits. TCE air contents were over tolerated levels, ranging from 50–150 ppm (275–825 mg/m<sup>3</sup>). The study is well presented with statistically significant results for trigeminal nerve impairment resulting from occupational exposures to TCE. Exposure potential to TCE is defined by urinary biomarkers, TCA, total trichloro-compounds, and TCOH. The study lacks information on atmospheric monitoring of TCE in this occupational setting.

### **D.1.1.3. Nerve Conduction Velocity Studies**

Nerve conduction latencies were also studied in two occupational studies by Triebig et al. (1982, 1983) using methods for measurement of nerve conduction which differ from most published studies, but the results indicate a potential impact on nerve conduction following occupational TCE exposure. There was no impact seen on latencies in the 1982 study, but a statistically significant response was observed in the latter study. The latter study, however, is confounded by multiple solvent exposures.

In Triebig et al. (1982), 24 healthy workers (20 males, 4 females) were exposed to TCE occupationally at three different plants. The ages ranged from 17–56, and length of exposure ranged from 1 month to 258 months (mean 83 months). TCE concentrations measured in air at work places ranged from 5–70 ppm (27–385 mg/m<sup>3</sup>). A control group of 144 healthy, complaint-free individuals were used to establish ‘normal’ responses on the nerve conduction studies. The matched control group consisted of twenty-four healthy nonexposed individuals (20 males, 4 females), chosen to match the subjects for age and sex. TCA, TCE, and trichloroethanol were measured in blood, and TCE and TCA were measured in urine. Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: MCV<sub>MAX</sub> (U): Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; dSCV (U): Distal NLG of mixed fibers of the N. ulnaris between finger V and the wrist joint; pSCV (U): Proximal NLG of sensory fibers of the N. medianus between finger V and Sulcus ulnaris; and dSCV (M): Distal NLG of sensory fibers of the N. medianus between finger III and the wrist joint. Data were analyzed using parametric and nonparametric tests, rank correlation, linear regression, with 5% error probability. Results show no statistically significant difference in nerve conduction velocities between the exposed and unexposed groups. This study has measured exposure data, but exposures/responses are not reported by dose levels.

Triebig et al. (1983) has a similar study design to the previous study (Triebig et al., 1982) in the tests used for measurement of nerve conduction velocities, and in the analysis of blood and urinary metabolites of TCE. However, in this study, subjects were exposed to a mixture of solvents, including TCE, specifically “ethanol, ethyl acetate, aliphatic hydrocarbons (gasoline), methyl ethyl ketone (MEK), toluene, and trichloroethene.” The exposed group consists of 66 healthy workers selected from a population of 112 workers. Workers were excluded based on polyneuropathy ( $n = 46$ ) and alcohol consumption ( $n = 28$ ). The control group consisted of 66 healthy workers with no exposures to solvents. Subjects were divided into three exposure groups based on length of exposure, as follows: 20 employees with “short-term exposure” (7–24 months); 24 employees with “medium-term exposure” (25–60 months); 22 employees with “long-term exposure” (over 60 months). TCA, TCE, and trichloroethanol were measured in blood, and TCE and TCA were measured in urine. Subjects were divided into exposure groups

*This document is a draft for review purposes only and does not constitute Agency policy.*

based on length of exposures, and results were compared for each exposure group to the control group. In this study, there was a dose-response relationship observed between length of exposure to mixed solvents and statistically significant reduction in nerve conduction velocities observed for the medium and long-term exposure groups for the ulnar nerve (NCV). Interpretation of this study is limited by the mixture of solvent exposure, with no results reported for TCE alone.

#### **D.1.2. Auditory Effects**

There are three large environmental studies reported which assessed the potential impact of TCE exposures through groundwater ingestion on auditory functioning. They present mixed results. All three studies were conducted on the population in the TCE Subregistry from the National Exposure Registry (NER) developed by the Agency for Toxic Substances Disease Registry (ATSDR). The two studies conducted by Burg et al. (1995; Burg and Gist, 1999) report an increase in auditory effects associated with TCE exposure, but the auditory endpoints were self reported by the population, as opposed to testing of measurable auditory effects in the subject population. The third of these studies, reported by ATSDR (2003) conducted measurements of auditory function on the subject population, but failed to demonstrate a positive relationship between TCE exposure and auditory effects. Results from these studies strongly suggest that children  $\leq 9$  years are more susceptible to hearing impairments from TCE exposure than the rest of the general population. These studies are described below.

Burg et al. (1995) conducted a study on registrants in the National Health Interview Survey (NHIS) TCE subregistry of 4,281 (4,041 living and 240 deceased) residents environmentally exposed to TCE via well water in Indiana, Illinois, and Michigan. Morbidity baseline data were examined from the TCE Subregistry from the NER developed by the ATSDR. Participants were interviewed in the NHIS, which consists of 25 questions about health conditions. Data were self reported via face-to-face interviews. Neurological endpoints were hearing and speech impairments. This study assessed the long-term health consequences of long-term, low-level exposures to TCE in the environment. The collected data were compared to the NHIS, and the National Household Survey on Drug Abuse. Poisson Regression analysis model was used for registrants 19 and older. The statistical analyses performed treated the NHIS population as a standard population and applied the age- and sex-specific period prevalence and prevalence rates obtained from the NHIS data to the corresponding age- and sex-specific denominators in the TCE Subregistry. This one-sample approach ignored sampling variability in the NHIS data because of the large size of the NHIS database when compared to the TCE Subregistry data file. A binomial distribution was assumed in estimating standard errors for the TCE Subregistry data. Weighted age- and sex-specific period prevalence and prevalence rates

*This document is a draft for review purposes only and does not constitute Agency policy.*

by using the person-weights were derived for the TCE subregistry. These “standard” rates were applied to the corresponding TCE Subregistry denominators to obtain expected counts in each age and sex combination. In the NHIS sample, 18% of the subjects were nonwhite. In the TCE Subregistry sample, 3% of the subjects were nonwhite. Given this discrepancy in the proportion of nonwhites and the diversity of races reported among the nonwhites in the TCE Subregistry, the statistical analyses included 3,914 exposed white TCE registrants who were alive at baseline. TCE registrants that were 9 years old or younger had a statistically significant increase in hearing impairment as reported by the subjects. The relative risk (RR) in this age group for hearing impairments was 2.13. The RR decreased to 1.12 for registrants aged 10–17 years and to 0.32 or less for all other age groups. As a result, the effect magnitude was lower for children 10–17 years and for all other age groups. The study reports a dose-response relationship, but the hearing effects are self-reported, and exposure data are modeled estimates.

Burg and Gist (1999) reported a study conducted on the same subregistry population described for Burg et al. (1995). It investigated intrasubregistry differences among 3,915 living members of the National Exposure Registry’s Trichloroethylene Subregistry (4,041 total living members). The participants’ mean age was 34 years (SD = 19.9 years), and included children in the registry. All registrants had been exposed to TCE through domestic use of contaminated well water. All were Caucasian. All registrants had been exposed to TCE through domestic use of contaminated well water; there were four exposure Subgroups, each divided into quartiles: (1) Maximum TCE measured in well water, exposure subgroups include 2–12 ppb; 12–60 ppb; 60–800 ppb; (2) Cumulative TCE exposure subgroups include <50 ppb, 50–500 ppb, 500–5,000 ppb, >5,000 ppb; (3) Cumulative chemical exposure subgroups include TCA, DCE, dichloroacetic acid (DCA), in conjunction with TCE, with the same exposure Categories as in # 2; and (4) Duration of exposure subgroups include <2 years, 2–5 years, 5–10 years, >10 years; 2,867 had TCE exposure of ≤50 ppb; 870 had TCE exposure of 51–500 ppb; 190 had TCE exposure of 501–5,000 ppb; 35 had TCE exposure >5,000 ppb. The lowest quartile was used as a control group. Interviews included occupational, environmental, demographic, and health information. A large number of health outcomes were analyzed, including speech impairment and hearing impairment. Statistical methods used include Logistic Regression and Odds Ratios. The primary purpose was to evaluate the rate of reporting health-outcome variables across exposure categories. The data were evaluated for an elevation of the risk estimates across the highest exposure categories or for a dose-response effect, while controlling for potential confounders. Estimated prevalence odds ratios for the health outcomes, adjusted for the potential confounders, were calculated by exponentiating the  $\beta$ -coefficients from the exposure variables in the regression equations. The standard error of the estimate was used to calculate 95% confidence intervals (CIs). The referent group used in the logistic regression models was

*This document is a draft for review purposes only and does not constitute Agency policy.*

the lowest exposure group. The results variables were modeled as dichotomous, binary dependent variables in the regression models. Nominal, independent variables were modeled, using dummy variables. The covariables used were sex, age, occupational exposure, education level, smoking history, and the sets of environmental subgroups. The analyses were restricted to persons 19 years of age or older when the variables of occupational history, smoking history, and education level were included. When the registrants were grouped by duration of exposure to TCE, a statistically significant association (adjusted for age and sex) between duration of exposure and reported hearing impairment was found. The prevalence odds ratios were 2.32 (95% CI: 1.18, 4.56) (>2 to <5 years); 1.17 (95% CI: 0.55, 2.49) (>5 to <10 years); and 2.46 (95% CI = 1.30, 5.02) (>10 years). Higher rates of speech impairment (although not statistically significant) were associated with maximum and cumulative TCE exposure, and duration of exposure. The study reports dose-response relationships, but the effects are self reported, and exposure data are estimates. No information was reported on presence or absence of additional solvents in drinking water.

ATSDR (2003) conducted a follow-up study to the TCE subregistry findings (Burg et al., 1995, 1999) and focused on the subregistry children. Of the 390 subregistry children ( $\leq 10$  years at time of original study), 116 agreed to participate. TCE exposure ranged from 0.4 to 5,000 ppb from the drinking water. The median TCE exposure for this subgroup was estimated to be 23 ppb per year of exposure. To further the hearing impairments reported in Burg et al. (1995, 1999), comprehensive auditory tests were conducted with the 116 children and compared to a control group of 182 children that was age-matched. The auditory tests consisted of a hearing screening (typanometry, pure tone and distortion product otoacoustic emissions [DPOAE]) and a more in-depth hearing evaluation for children that failed the initial screening. Ninety percent of the TCE-exposed children passed the typanometry and pure tone tests, and there were no significant differences between control and TCE-exposed groups. Central auditory processing tests were also conducted and consisted of a test for acoustic reflexes and a screening test for auditory processing disorders (SCAN). The acoustic reflex tested the ipsilateral and contralateral auditory pathway at 1,000 Hz for each ear. In this test, each subject hears the sound frequency and determines if the sound causes the stapedius muscle to tighten the stapes (normal reflex to noise). Approximately 20% of the children in the TCE subregistry and 5–7% in the controls exhibited an abnormal acoustic reflex, and this increased abnormality in the test was a significant effect ( $p = 0.003$ ). No significant effects were noted in the SCAN tests. The authors concluded that the significant decrease in the acoustic reflex for the TCE subregistry children is reflective of potential abnormalities in the middle ear, which may reflect abnormalities in lower brainstem auditory pathway function. Lack of effects with the pure tone and typanometry tests suggests that the cochlea is not affected by TCE exposure.

*This document is a draft for review purposes only and does not constitute Agency policy.*

Although auditory function was not directly measured, Rasmussen et al. (1993b) used a psychometric test to measure potential auditory effects of TCE exposure in an environmental study. Results from 96 workers exposed to TCE and other solvents were presented in this study. The workers were divided into three exposure groups: low, medium, and high. Details of the exposure groups and exposure levels are provided in Table 4-21 (under study description of Rasmussen et al., 1993b). Three auditory-containing tasks were included in this study, but only the acoustic motor function test could be used for evaluation of auditory function. In the acoustic motor function test, high and low frequency tones were generated and heard through a set of earphones. Each individual then had to imitate the tones by knocking on the table using the flat hand for a low frequency and using a fist for a high frequency. A maximal score of 8 could be achieved through this test. The tones were provided in either a set of 1 or 3 groups. In the one group acoustic motor function test, the average score for the low exposure group was 4.8 in comparison to 2.3 in the high exposure group. Similar decrements were noted in the 3 group acoustic motor function test. A significant association was reported for TCE exposure and performance on the one group acoustic motor function test ( $p < 0.05$ ) after controlling for confounding variables.

### **D.1.3. Vestibular Effects**

The data linking acute TCE exposure with transient impairment of vestibular function are quite strong based on human chamber studies, occupational exposure studies, and laboratory animal investigations. It is clear from the human literature that these effects can be caused by exposures to TCE, as they have been reported extensively in the literature.

The earliest reports of neurological effects resulting from TCE exposures focused on subjective symptoms, such as headaches, dizziness, and nausea. These symptoms are subjective and self-reported, and, therefore, offer no quantitative measurement of cause and effect. However, there is little doubt that these effects can be caused by exposures to TCE, as they have been reported extensively in the literature, resulting from occupational exposures (Grandjean et al., 1955; Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith, 1970), environmental exposures (Hirsch et al., 1996), and in chamber studies (Stewart et al., 1970; Kylin et al., 1967). These studies are described below in more detail.

Grandjean et al. (1955) reported on 80 workers exposed to TCE from 10 different factories of the Swiss mechanical engineering industry. TCE air concentrations varied from 6–1,120 ppm (33–6,200 mg/m<sup>3</sup>) depending on time of day and proximity to tanks, but mainly averaged between 20–40 ppm (100–200 mg/m<sup>3</sup>). Urinalysis (TCA) varied from 30 mg/L to 300 mg/L. This study does not include an unexposed referent group, although prevalences of self-reported symptoms or neurological changes among the higher-exposure group are compared

*This document is a draft for review purposes only and does not constitute Agency policy.*



to the lower-exposure group. Workers were classified based on their exposures to TCE and there were significant differences ( $p = 0.05$ ) in the incidence of neurological disorder between Groups I (10–20 ppm), II (20–40 ppm; 110–220 mg/m<sup>3</sup>) and III (>40 ppm; 220 mg/m<sup>3</sup>). Thirty-four percent of the workers had slight or moderate psycho-organic syndrome; 28% had neurological changes. Approximately 50% of the workers reported incidences of vertigo and 30% reported headaches (primarily an occasional and/or minimal disorder). Based on TCA eliminated in the urine, results show that subjective, vegetative, and neurological disorders were more frequent in Groups II (40–100 mg/L) and III (101–250 mg/L) than in Group I (10–39 mg/L). Statistics do support a dose-effect relationship between neurological effects and TCE exposure, but exposure data are questionable.

Liu et al. (1988) evaluated the effects of occupational TCE exposure on 103 factory workers in Northern China. The workers (79 men, 24 women) were exposed to TCE during vapor degreasing production or operation. An unexposed control group of 85 men and 26 women was included for comparison. Average TCE exposure was mostly at less than 50 ppm (275 mg/m<sup>3</sup>). The concentration of breathing zone air during entire shift was measured by diffusive samplers placed on the chest of each worker. Subjects were divided into three exposure groups; 1–10 ppm (5.5–55 mg/m<sup>3</sup>), 11–50 ppm (60–275 mg/m<sup>3</sup>) and 51–100 ppm (280–550 mg/m<sup>3</sup>). Results were based on a self-reported subjective symptom questionnaire. The frequency of subjective symptoms, such as nausea, drunken feeling, light-headedness, floating sensation, heavy feeling of the head, forgetfulness, tremors and/or cramps in extremities, body weight loss, changes in perspiration pattern, joint pain, and dry mouth (all  $\geq 3$  times more common in exposed workers); reported as ‘prevalence of affirmative answers’, was significantly greater in exposed workers than in unexposed ( $p < 0.01$ ). “*Bloody strawberry jam-like feces*” was borderline significant in the exposed group and “*frequent flatus*” was statistically significant. Dose-response relationships were established (but not statistically significant) for symptoms. Most workers were exposed below 10 ppm, and some at 11–50 ppm. The differences in exposure intensity between men and women was of borderline significance ( $0.05 < p < 0.10$ ). The study appears to be well done, although the self reporting of symptoms and the ‘prevalence of affirmative answers’ metric is not standard practice.

Rasmussen et al. (1986) conducted a cross-sectional study on 368 metal degreasers working in various factories in Denmark (industries not specified) with chlorinated solvents. The control group consisted of 94 randomly selected semiskilled metal workers from same area. The mean age was 37.7 (range: 17–65+). Neurological symptoms of the subjects were assessed by questionnaire. The workers were categorized into four groups as follows: (1) currently working with chlorinated solvents ( $n = 171$ ; average duration: 7.3 years, 16.5 hours/week; 57% TCE and 37% 1,1,1-trichloroethane), (2) currently working with other solvents ( $n = 131$ ;

*This document is a draft for review purposes only and does not constitute Agency policy.*

petroleum, gasoline, toluene, xylene), (3) previously (1–5 years.) worked with chlorinated or other solvents ( $n = 66$ ), (4) never worked with organic solvents ( $n = 94$ ). A dose-response relationship was observed between exposure to chlorinated solvents and chronic neuropsychological symptoms including vestibular system effects such as dizziness ( $p < 0.005$ ), and headache ( $p < 0.01$ ). The authors indicated that TCE exposure resulted in the most overall symptoms. Significant associations were seen between previous exposure and consumption of alcohol with chronic neuropsychological symptoms. Results are confounded by exposures to additional solvents.

Smith (1970) conducted an occupational study on 130 workers (108 males, 22 females) exposed to TCE (industry not reported). The control group consisted of 63 unexposed men working at the same factories matched by age, marital status and other nonspecified criteria. A referent group was included and consisted of 112 men and women exposed to low concentration of lead and matched to the TCE exposed group in age and sex distribution. Seventy-three out of 130 workers (56.2%) reported dizziness and 23 workers reported having headaches (17.7%). The number of complaints reported by subjects was greater for those with 60 mg/L or greater TCA than for those with less than 60 mg/L TCA. There was no difference in the number of symptoms reported between those with shorter durations of exposure and those with longer durations of exposure. No statistics were reported.

Hirsh et al. (1996) evaluated the vestibular effects of an environmental exposure to TCE in Roscoe, IL residents. A medical questionnaire was mailed to 103 residents of Roscoe with 100% response. These 103 and an additional 15 residents, not previously surveyed, brought the subject population to 118 residents. During the course of testing, 12 subjects (young children and uncooperative patients) were excluded bringing the total number of subjects to 106 all of whom were in the process of taking legal action against the company whose industrial waste was assumed to be the source of the polluting TCE. This was a case series report with no controls. Random testing of the wells between 1983–84 revealed groundwater in wells to have levels of TCE between 0 to 2,441 ppb. The distance of residence from contaminated well was used to estimate exposure level. Sixty-six subjects (62%) complained of headaches at the time of evaluation. Diagnosis of TCE-induced cephalgia was considered credible for 57 patients (54%). Forty-seven of these had a family history of headaches. Retrospective TCE level of well water or well's distance from the industrial site analysis did not correlate with the occurrence of possibly-TCE induced headaches. This study shows a general association between headaches and exposure to TCE in drinking water wells. There were no statistics to support a dose-response relationship. All subjects were involved in litigation.

Stewart et al. (1970) evaluated vestibular effects in 13 subjects who were exposed to TCE vapor 100 ppm ( $550 \text{ mg/m}^3$ ) and 200 ppm ( $1,100 \text{ mg/m}^3$ ) for periods of 1 hour to a 5-day work

*This document is a draft for review purposes only and does not constitute Agency policy.*

week. Experiments 1–7 were for a duration of 7 hours with a mean TCE concentration of 198–200 ppm (1,090–1,100 mg/m<sup>3</sup>). Experiments 8 and 9 exposed subjects to 190–202 ppm (1,045–1,110 mg/m<sup>3</sup>) TCE for a duration of 3.5 and 1 hour, respectively. Experiment 10 exposed subjects to 100 ppm (550 mg/m<sup>3</sup>) TCE for 4 hours. Experiments 2–6 were carried out with the same subjects over 5 consecutive days. Gas chromatography of expired air was measured. There were no self controls. Subjects reported symptoms of lightheadedness, headache, eye, nose, and throat irritation. Prominent fatigue and sleepiness by all were reported above 200 ppm (1,100 mg/m<sup>3</sup>). There were no quantitative data or statistics presented regarding dose and effects of neurological symptoms.

Kylin et al. (1967) exposed 12 volunteers to 1,000 ppm (5,500 mg/m<sup>3</sup>) TCE for 2 hours in a 1.5 × 2 × 2 meters chamber. Volunteers served as their own controls since 7 of the 12 were pretested prior to exposure and the remaining 5 were post-tested days after exposure. Subjects were tested for optokinetic nystagmus, which was recorded by electronystagmography, that is, “the potential difference produced by eye movements between electrodes placed in lateral angles between the eyes.” Venous blood was also taken from the volunteers to measure blood TCE levels during the vestibular task. The authors concluded that there was an overall reduction in the limit (“fusion limit”) to reach optokinetic nystagmus when individuals were exposed to TCE. Reduction of the “fusion limit” persisted for up to 2 hours after the TCE exposure was stopped and the blood TCE concentration was 0.2 mg/100 mL.

#### **D.1.4. Visual Effects**

Kilburn (2002a) conducted an environmental study on 236 people exposed to TCE in groundwater in Phoenix, AZ. Details of the TCE exposure and population are described earlier in Section D.1.1.1 (see Kilburn [2002a]). Among other neurological tests, the population and 161 nonexposed controls was tested for color discrimination using the desaturated Lanthony 15-hue test, which can detect subtle changes in color vision deficiencies. Color discrimination errors were significantly increased in the TCE exposed population ( $p < 0.05$ ) with errors scores averaging 12.6 in the TCE exposed in comparison to 11.9 in the control group. This study shows statistically significant differences in visual response between exposed and nonexposed subjects exposed environmentally. Estimates of TCE concentrations in drinking water to individual subjects are lacking.

Reif et al. (2003) conducted a cross sectional environmental study on 143 residents of the Rocky Mountain Arsenal community of Denver whose water was contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986. The residents were divided into three groups based on TCE exposure with the lowest exposure group at <5 ppb, the medium exposure group at 5 to 15 ppb and the high exposure group defined as

>15 ppb TCE. Visual performance was measured by two different contrast sensitivity tests (C and D) and the Benton visual retention test. In the two contrast sensitivity tests, there was a 20 to 22% decrease in performance between the low and high TCE exposure groups and approached statistical significance ( $p = 0.06$  or  $0.07$ ). In the Benton visual retention test, which measures visual perception and visual memory, scores, dropped by 10% from the lowest exposure to the highest TCE exposure group and was not statistically significant. It should be noted that the residents were potentially exposed to multiple solvents including TCE and a nonexposed TCE group was not included in the study. Additionally, modeled exposure data are only a rough estimate of actual exposures, and possible misclassification bias associated with exposure estimation may limit the sensitivity of the study.

Rasmussen et al. (1993b) conducted a cross-sectional study on 96 metal workers, working in degreasing at various factories in Denmark (industries not specified) with chlorinated solvents. These subjects were identified from a larger cohort of 240 workers. Details of the exposure groups and TCE exposure levels are presented in Section D.1.1.1 (under Rasmussen et al., 1993c). Neuropsychological tests including the visual gestalts (test of visual perception and retention) and the stone pictures test (test of visual learning and retention) were administered to the metal workers. In the visual gestalts test, cards with a geometrical figure containing four items were presented and workers had to redraw the figure from memory immediately (learning phase) after presentation and after 1 hour (retention phase). In the learning phase, the figures were redrawn until the worker correctly drew the figure. The number of total errors significantly increased from the low group (3.4 errors) to the high exposure group (6.5 errors;  $p = 0.01$ ) during the learning phase (immediate presentation). Similarly, during the retention phase of this task (measuring visual memory), errors significantly increased from an average of 3.2 in the low group to 5.9 in the high group ( $p < 0.001$ ). In the stone pictures test, slides of 10 stones (different shapes and sizes) were shown and the workers had to identify the 10 stones out of a lineup of 25 stones. There were no significant changes in this task, but the errors increased from 4.6 in the low exposure group to 6.3 in the high exposure group during the learning phase of this task. Although this study identifies visual performance deficits, a control group (no TCE exposure) was not included in this study and the presented results may actually underestimate visual deficits from TCE exposure.

Troster and Ruff (1990) presented case studies conducted on two occupationally exposed workers to TCE and included a third case study on an individual exposed to 1,1,1-trichloroethane. Case #1 was exposed to TCE (concentration unknown) for 8 months and Case #2 was exposed to TCE over a 3-month period. Each patient was presented with a visual-spatial task (Ruff-Light Trail Learning test as referenced by the authors). Both of the individuals exposed to TCE were unable to complete the visual-spatial task and took the

maximum number of trials (10) to attempt to complete the visual task. A control group of 30 individuals and the person exposed to 1,1,1-trichloroethane were able to complete this task accordingly. The lack of quantitative exposure data and a small sample size severely limits the study and does not allow for statistical comparisons.

Vernon and Ferguson (1969) exposed eight male volunteers (ages 21–30) to 0, 100, 300, and 1,000-ppm TCE for 2 hours. Each individual was exposed to all TCE concentrations and a span of at least 3 days was given between exposures. The volunteers were presented with six visuo-motor tests during the exposure sessions. When the individuals were exposed to 1,000-ppm TCE (5,500 mg/m<sup>3</sup>), significant abnormalities were noted in depth perception as measured by the Howard-Dolman test ( $p < 0.01$ ), but no effects on the flicker fusion frequency test (threshold frequency at which the individual sees a flicker as a single beam of light) or on the form perception illusion test (volunteers presented with an illusion diagram). This is one of the earliest chamber studies of TCE. This study included only healthy young males, is of a small size, limiting statistical power, and reports mixed results on visual testing following TCE exposure.

#### **D.1.5. Cognition**

There is a single environmental study in the literature that presents evidence of a negative impact on intelligence resulting from TCE exposure. Kilburn and Warshaw (1993—study details in Section D.1.1.1) evaluated the effects on cognition for 544 Arizona residents exposed to TCE in well-water. Subjects were recruited and categorized into three groups. Exposed Group 1 consisted of 196 family members with cancer or birth defects. Exposed Group 2 consisted of 178 individuals from families without cancer or birth defects; and exposed Group 3 included 170 parents whose children had birth defects and rheumatic disorders. Sixty-eight referents were used as a comparison group for the clinical memory tests. Several cognitive tests were administered to these residents in order to test memory recall skills and determine if TCE exposure resulted in memory impairment. Working or short-term memory skills were tested by asking each individual to recall two stories immediately after presentation (verbal recall) and also draw three diagrams immediately after seeing the figures (visual recall). Additionally, a digit span test where increasing numbers of digits were presented and then the subject had to recall the digits was conducted to the extent of the short-term memory. Exposed subjects had lower intelligence scores and there were significant impairments in verbal recall ( $p = 0.001$ ), visual recall ( $p = 0.03$ ) and with the digit span test ( $p = 0.07$ ). Significant impairment in short-term memory as measured by three different cognitive test was correlated with TCE exposure. Lower intelligence scores ( $p = 0.0001$ ) as measured by the Culture Fair IQ test may be a possible confounder in these findings. Additionally, the large range of TCE concentrations

*This document is a draft for review purposes only and does not constitute Agency policy.*

(6–500 ppb) and exposure durations (1 to 25 years) and overall poor exposure characterization precludes a no-observed-adverse-effect level (NOAEL)/lowest-observed-adverse-effect level (LOAEL) from being estimated from this study on cognitive function.

Rasmussen et al. (1993a, b) and Troster and Ruff (1990) present results of positive findings in occupational studies for cognitive effects of TCE. Rasmussen et al. (1993a) reported an historical cohort study conducted on 96 metal degreasers, identified 2 years previously and were selected from a population of 240 workers from 72 factories in Denmark. They reported psychoorganic syndrome, a mild syndrome of dementia characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative, was increased in the three exposure groups. The medium and high exposure groups were compared with the low exposure group. Neuropsychological tests included WAIS (original version, Vocabulary, Digit Symbol, Digit Span), Simple Reaction Time, Acoustic-motor function (Luria), Discriminatory attention (Luria), Sentence Repetition, Paced Auditory Serial Addition Test (PASAT), Text Repetition, Rey's Auditory Verbal Learning, Visual Gestalts, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, and Mira. The prevalence of psychoorganic syndrome was 10.5% in low exposure group; 38.9% in medium exposure group; 63.4% in high exposure group. ( $\chi^2$  trend analysis: low vs. medium exposure  $\chi^2 = 11.0$ ,  $p$  value  $<0.001$ ; low vs. high exposure  $\chi^2 = 19.6$ ,  $p$ -value  $<0.001$ .) Psychoorganic syndrome increased with age ( $p < 0.01$ ). Age was strongly correlated with exposure.

Rasmussen et al. (1993b) used a series of cognitive tests to measure effects of occupational TCE exposure. Short-term memory and retention following an latency period of one hour was evaluated in several tests including a verbal recall (auditory verbal learning test), visual gestalts, visual recall (stone pictures), and the digit span test. Significant cognitive performance decreases were noted in both short-term memory and memory retention. In the verbal recall test immediate memory and learning were significantly decreased ( $p = 0.03$  and  $0.04$ , respectively). No significant effects were noted for retention following a one hour latency period was noted. Significant increases in errors were noted in both the learning ( $p = 0.01$ ) and memory ( $p < 0.001$ ) phases for the visual gestalts test. No significant effects were found in the visual recall test in either the learning or memory phases or in the digit span test. As a result, there were some cognitive deficits noted in TCE-exposed individuals as measured through neuropsychological tests.

Troster and Ruff (1990) provides additional supporting evidence in an occupational study for cognitive impairment, although the results reported in a qualitative fashion are limited in their validity. In the two case studies that were exposed to TCE, there were decrements (no statistical analysis performed) in cognitive performance as measured in verbal and visual recall tests that

were conducted immediately after presentation (learning phase) and one hour after original presentation (retention/memory phase).

Triebig et al. (1977b) presents findings of no impairment of cognitive ability resulting from TCE exposure in an occupational setting. This study was conducted on 8 subjects occupationally exposed to TCE. Subjects were 7 men and 1 woman with an age range from 23–38 years. Measured TCE in air averaged 50 ppm (260 mg/m<sup>3</sup>). Length of occupational exposure was not reported. There was no control group. Results were compared after exposure periods, and compared to results obtained after periods removed from exposure. TCA and TCE metabolites in urine and blood were measured. The testing consisted of the Syndrome Short Test, which consists of nine subtests through which amnesic and simple perceptible and cognitive functional deficits are detected; the “Attention Load Test” or “d2 Test” from Brickenkamp is a procedure that measures attention, concentration, and stamina. Number recall test, letter recall test, the “Letter Reading Test,” “Word Reading Test.” Data were assessed using Wilcoxon and Willcox nonparametric tests. Due to the small sample size a significance level of 1% was used. The concentrations of TCE, trichloroethanol, and TCA in the blood and total TCE and total TCA elimination in the urine were used to assess exposure in each subject. The mean values observed were 330 mg trichloroethanol and 319 mg TCA/g creatinine, respectively, at the end of a work shift. The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period. The small sample size may limit the sensitivity of the study.

Salvini et al. (1971), Gamberale et al. (1976), and Stewart et al. (1970) reported positive findings for the impairment of cognitive function following TCE exposures in chamber studies. Salvini et al. (1971) reported a controlled exposure study conducted on six male university students. TCE concentration was 110 ppm (550 mg/m<sup>3</sup>) for 4-hour intervals, twice per day. Each subject was examined on two different days, once under TCE exposure, and once as self controls, with no exposure. Two sets of tests were performed for each subject corresponding to exposure and control conditions. The test battery included a perception test with tachoscopic presentation, the Wechsler memory scale test, a complex reaction time test, and a manual dexterity test. Statistically significant results were observed for perception tests learning ( $p < 0.001$ ), mental fatigue ( $p < 0.01$ ), subjects ( $p < 0.05$ ); and CRT learning ( $p < 0.01$ ), mental fatigue ( $p < 0.01$ ), subjects ( $p < 0.05$ ). This is controlled exposure study with measured dose (110 ppm; 600 mg/m<sup>3</sup>) and clear, statistically significant impact on neurological functional domains. However, it only assesses acute exposures.

Gamberale et al. (1976) reported a controlled exposure study conducted on 15 healthy men aged 20–31 yrs old, employed by the Department of Occupational Medicine in Stockholm, Sweden. Controls were within subjects (15 self-controls), described above. Test used included

*This document is a draft for review purposes only and does not constitute Agency policy.*

reaction time (RT) Addition and short term memory using an electronic panel. Subjects also assessed their own conditions on a 7-point scale. Researchers used a repeated measures analysis of variance (ANOVA) for the 4 performance tests based on a  $3 \times 3$  Latin square design. In the short-term memory test (version of the digit span test), a series of numbers lasting for one second was presented to the subject. The volunteer then had to reproduce the numerical sequence after a latency period (not specified). No significant effect on the short-term memory test was observed with TCE exposure in comparison to air exposure. Potential confounders from this study include repetition of the same task for all exposure conditions, volunteers served as their own controls, and TCE exposure preceded air exposure in two of the three exposure experimental designs. This is a well controlled study of short term exposures with measured TCE concentrations and significant response observed for cognitive impairment.

Additional qualitative support for cognitive impairment is provided by Stewart et al. (1970). This was a controlled exposure study conducted on 13 subjects in 10 experiments, which consisted of ten chamber exposures to TCE vapor of 100 ppm ( $550 \text{ mg/m}^3$ ) and 200 ppm ( $1,100 \text{ mg/m}^3$ ) for periods of 1 hour to a 5-day work week. Experiments 1–7 were for 7 hours with a mean TCE concentration of 198–200 ppm ( $1,090\text{--}1,100 \text{ mg/m}^3$ ). Experiments 8 and 9 exposed subjects to 190–202 ppm ( $1,045\text{--}1,110 \text{ mg/m}^3$ ) TCE for a duration of 3.5 and 1 hour, respectively. Experiment 10 exposed subjects to 100 ppm ( $550 \text{ mg/m}^3$ ) TCE for 4 hours. Experiments 2–6 were carried out with the same subjects over 5 consecutive days. Gas chromatography of expired air was measured. There were no self controls. All had normal neurological tests during exposure, but 50% reported greater mental effort was required to perform a normal modified Romberg test on more than one occasion. There were no quantitative data or statistics presented regarding dose and effects of neurological symptoms.

Two chamber studies conducted by Triebig et al. (1976, 1977a) report no impact of TCE exposure on cognitive function. Triebig et al. (1976) was a controlled exposure study conducted on 7 healthy male and female students (4 females, 3 males) exposed for 6 hours/day for 5 days to 100 ppm ( $550 \text{ mg/m}^3$  TCE). The control group was 7 healthy students (4 females, 3 males) exposed to hair care products. This was assumed as a zero exposure, but details of chemical composition were not provided. Biochemical and psychological testing was conducted at the beginning and end of each day. Biochemical tests included TCE, TCA, and trichloroethanol in blood. Psychological tests included the d2 test, which was an attention load test; the short test (as characterized in the translated version of Treibig, 1976) is used to record patient performance with respect to memory and attention; daily Fluctuation Questionnaire measured the difference between mental states at the start of exposure and after the end of exposure is recorded; The MWT-A is a repeatable short intelligence test; Culture Fair Intelligence Test (CFT-3) is a nonverbal intelligence test that records the rather “fluid” part of intelligence, that is, finding

*This document is a draft for review purposes only and does not constitute Agency policy.*



solution strategies; Erlanger Depression Scale. Results were not randomly distributed. The median was used to describe the mean value. Regression analyses were conducted. In this study the TCE concentrations in blood reported ranged from 4 to 14 µg/mL. A range of 20 to 60 µg/mL was obtained for TCA in the blood. There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. The biochemical data did demonstrate subjects' exposures. This is a well controlled study with excellent exposure data, although the small sample size may have limited sensitivity.

Triebig et al. (1977a) is an additional report on the seven exposed subjects and seven controls evaluated in Triebig et al. (1976). Additional psychological testing was reported. The testing included the Syndrome Short Test, which consists of nine subtests, described above. Statistics were conducted using Whitney Mann. Results indicated the anxiety values of the placebo random sample group dropped significantly more during the course of testing ( $p < 0.05$ ) than those of the active random sample group. No significantly different changes were obtained with any of the other variables. Both these studies were well controlled with excellent exposure data, which may provide some good data for establishing a short term NOAEL. The small sample size may have limited the sensitivity of the study.

Additional reports on the impairment of memory function as a result of TCE exposures have been reported, and provide additional evidence of cognitive impairment. The studies by Chalupa et al. (1960), Rasmussen et al. (1986, 1993b), and Troster and Ruff (1990) report impairment of memory resulting from occupational exposures to TCE. Kilburn and Warshaw (1993) and Kilburn (2002a) report impairment of memory following environmental exposures to TCE. Salvini et al. (1971) reports impairment of memory in a chamber study, although Triebig et al. (1976) reports no impact on memory following TCE exposure in a chamber study.

#### **D.1.6. Psychomotor Effects**

There is evidence in the literature that TCE can have adverse psychomotor effects in humans. The effects of TCE exposure on psychomotor response have been studied primarily as the impact on RTs, which provide a quantitative measure of the impact TCE exposure has on motor skills. Studies on motor dyscoordination resulting from TCE exposure are more subjective, but provide additional evidence that TCE may cause adverse psychomotor effects. These studies are described below.

##### **D.1.6.1. Reaction Time**

There are several reports in the literature that report an increase in reaction times following exposures to TCE. The best evidence for TCE exposures causing an increase in choice reaction times comes from environmental studies by Kilburn (2002a), Kilburn and Warshaw

*This document is a draft for review purposes only and does not constitute Agency policy.*

(1993), Reif et al. (2003), and Kilburn and Thornton (1996), which were all conducted on populations which were exposed to TCE through groundwater contaminated as the result of environmental spills. Kilburn (2002a—study details described in Section D.1.1) evaluated reaction times in a Phoenix, Arizona population exposed to TCE through groundwater. Volunteers were tested for response rates in the simple reaction time (SRT) and 2 choice reaction time (CRT) tests. Various descriptive statistics were used, as well as analysis of covariance (ANCOVA) and a step-wise adjustment of demographics. The principal comparison, between the 236 exposed persons and the 161 unexposed regional controls, revealed significant differences ( $p < 0.05$ ) indicating that SRTs and CRTs were delayed. Balance was also abnormal with excessive sway speed (eyes closed), but this was not true when both eyes were open. This study shows statistically significant differences in psychomotor responses between exposed and nonexposed subjects exposed environmentally. However, it is limited by poor exposure characterization.

Kilburn and Warshaw (1993; study details described in Section D.1.1.1) evaluated reaction times in 170 Arizona residents exposed to TCE in well water. A referent group of 68 people was used for comparison. TCE concentration was from 6 to 500 ppb and exposure ranged from 1 to 25 years. SRT was determined by presenting the subject a letter on a computer screen and measuring the time (in milliseconds [msec]) it took for the person to type that letter. SRT significantly increased from  $281 \pm 55$  msec to  $348 \pm 96$  msec in TCE-exposed individuals ( $p < 0.0001$ ). Similar increases were reported for CRT where subjects were presented with two different letters and required to make a decision as to which letter key to press. CRT of the exposed subjects was 93 msec longer in the third trial ( $p < 0.0001$ ) than referents. It was also longer in all trials, and remained significantly different after age adjustment. This study shows statistically significant differences for neurological test results between subjects environmentally exposed and nonexposed to TCE, but is limited by poor exposure data on individual subjects given the ecological design of this study. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Kilburn and Thornton (1996) conducted an environmental study that attempts to use reference values from two control groups in assessing neurological responses for chemically exposed subjects using neurophysiological and neuropsychological testing on three groups. Group A included randomly selected registered voters from Arizona and Louisiana with no exposure to TCE:  $n = 264$  unexposed volunteers aged 18–83. Group B included volunteers from California  $n = 29$  (17 males and 12 females) that were used to validate the equations; Group C included those exposed to TCE and other chemicals residentially for 5 years or more  $n = 237$ . Group (A), was used to develop the regression equations for SRT and choice reaction time (CRT). A similarly selected comparison group B was used to validate the equations. Group C,

*This document is a draft for review purposes only and does not constitute Agency policy.*

the exposed population, was submitted to SRT and CRT tests ( $n = 237$ ) and compared to the control groups. All subjects were screened by a questionnaire. Reaction speeds were measured using a timed computer visual-stimulus generator. No exposure data were presented. The Box-Cox transformation was used for dependent variables and independent variables. They evaluated graphical methods to study residual plots. Cook's distance statistic was used as a measure of influence to exclude outliers with undue influence and none of the data were excluded. Lack-of-fit test was performed on Final model and F statistic was used to compare estimated error to lack-of-fit component of the model's residual sum of squared error. Final models were validated using group B data and paired t-test to compare observed values for SRT and CRT. F statistic was used to test the hypothesis that parameter estimates obtained with group B were equal to those of Group A, the model. The results are as follows: Group A: SRT = 282 ms; CRT = 532 ms. Group B: SRT = 269 ms; CRT = 531 ms. Group C: SRT = 334 ms; CRT = 619 ms. TCE exposure produced a step increase in reaction times (SRT and CRT). The coefficients from Group A were valid for group B. The predicted value for SRT and for CRT, plus 1.5 SDs selected 8% of the model group as abnormal. The model produced consistent measurement ranges with small numerical variation. This study is limited by lack of any exposure data, and does not provide statistics to demonstrate dose-response effects.

Kilburn (2002a) conducted an environmental study on 236 residents chronically exposed to TCE-associated solvents in the groundwater resulting from a spill from a microchip plant in Phoenix, AZ. Details of the TCE exposure and population are described earlier in Section D.1.1.1 (see Kilburn [2002a]). The principal comparison, between the 236 exposed persons and the 161 unexposed regional controls, revealed significant differences indicating that SRTs and choice reaction times (CRTs) were increased. SRTs significantly increased from  $283 \pm 63$  msec in controls to  $334 \pm 118$  msec in TCE exposed individuals ( $p < 0.0001$ ). Similarly, CRTs also increased from  $510 \pm 87$  msec to  $619 \pm 153$  msec with exposure to TCE ( $p < 0.0001$ ). This study shows statistically significant differences in psychomotor responses as measured by reaction times between TCE-exposed and nonexposed subjects. Estimates of TCE concentrations in drinking water to individual subjects were not reported in the paper. Since the TCE exposure ranged from 0.2 to over 10,000 ppb in well water, it is not possible to determine a NOAEL for increased reaction times through this study. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Reif et al. (2003) conducted a cross sectional study on 143 residents of the Rocky Mountain Arsenal (RMA) community of Denver exposed environmentally to drinking water contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986. The referent group was at the lowest estimated exposure concentration (<5 ppb). The socioeconomic profile of the participants closely resembled those of the community in general.

*This document is a draft for review purposes only and does not constitute Agency policy.*

“A total of 3393 persons was identified through the census, from which an age- and gender-stratified sample of 1267 eligible individuals who had lived at their current residence for at least 2 years was drawn. Random selection was then used to identify 585 persons from within the age-gender strata, of whom 472 persons aged 2–86 provided samples for biomonitoring. Neurobehavioral testing was conducted on 204 adults who lived in the RMA exposure area for a minimum of 2 years. Among the 204 persons who were tested, 184 (90.2%) lived within the boundaries of the LWD and were originally considered eligible for the current analysis. Therefore, participants who reported moving into the LWD after 1985 were excluded from the total of 184, leaving 143 persons available for study.” An elaborate hydraulic simulation model (not validated) was used in conjunction with a geographic information system (GIS) to model estimates of residential exposures to TCE. The TCE concentration measured in community wells exceeded the MCL of 5 ppb in 80% of cases. Approximately 14% of measured values exceeded 15 ppb. Measured values were used to model actual exposure estimates based on distance of residences from sampled wells. The estimated exposure for the high exposure group was >15 ppb; the estimate for the low exposure referent group was <5 ppb. The medium exposure group was estimated at exposures  $5 < x < 15$  ppb TCE. The test battery consisted of the Neurobehavioral Core Test Battery (NCTB), which consists of 7 neurobehavioral tests including simple reaction time. Results were assessed using the Multivariate Model. Results were statistically significant ( $p < 0.04$ ) for the simple reaction time tests. The results are confounded by exposures to additional solvents and modeled exposure data, which while highly technical, are still only a rough estimate of actual exposures, and may limit the sensitivity of the study.

Gamberale et al. (1976) conducted a controlled exposure (chamber) study on 15 healthy men aged 20–31 yrs old, employed by the Department of Occupational Medicine in Stockholm, Sweden. Controls were within subjects (15 self-controls). Subjects were exposed to TCE for 70 minutes via a breathing valve to  $540 \text{ mg/m}^3$  (97 ppm),  $1,080 \text{ mg/m}^3$  (194 ppm), and to ordinary atmospheric air (0 ppm). Sequence was counterbalanced between the 3 groups, days, and exposure levels. Concentration was measured with a gas chromatographic technique every third minute for the first 50 minutes, then between tests thereafter. Test used were RT addition, simple RT, choice RT and short term memory using an electronic panel. Subjects also assessed their own conditions on a 7 point scale. The researchers performed Friedman two-way analysis by ranks to evaluate differences between the 3 conditions. The results were nonsignificant when tested individually, but significant when tested on the basis of six variables. Nearly half of the subjects could distinguish exposure/nonexposure. Researchers performed ANOVA for the four performance tests based on a  $3 \times 3$  Latin square design with repeated measures. In the RT-Addition test the level of performance varied significantly between the different exposure conditions ( $F[2.24] = 4.35; p < 0.05$ ) and between successive measurement occasions

*This document is a draft for review purposes only and does not constitute Agency policy.*

( $F[2.24] = 19.25; p < 0.001$ ). The level of performance declined with increased exposure to TCE, whereas repetition of the testing led to a pronounced improvement in performance as a result of the training effect. No significant interaction effects were observed between exposure to TCE and training. This is a good study of short term exposures with measured TCE concentrations and significant response observed for reaction time.

Gun et al. (1978) conducted an occupational study on 8 TCE-exposed workers who operated degreasing baths in two different plants. Four female workers were exposed to TCE only in one plant and four female workers were exposed to TCE and nonhalogenated hydrocarbon solvents in the second plant. The control group ( $n = 8$ ) consisted of 4 female workers from each plant who did not work near TCE. Each worker worked 2 separate 4-hour shifts daily, with one shift exposed to TCE and the second 4-hour shift not exposed. Personal air samples were taken continuously over separate 10-minute sessions. Readings were taken every 30 seconds. Eight-choice reaction times were carried out in four sessions; at the beginning and end of each exposure to TCE or TCE + solvents; a total of 40 reaction time trials were completed. TCE concentrations in the TCE only plant 1 (148–418 ppm [800–2,300 mg/m<sup>3</sup>]) were higher than in the TCE + solvent plant 2 (3–87 ppm (16–480 mg/m<sup>3</sup>)). Changes in choice reaction times (CRT) were compared to level of exposure. The TCE only group showed a mean increase in reaction time, with a probable cumulative effect. In the TCE + solvent group, mean reaction time shortened in Session 2, then increased to be greater than at the start. Both control groups showed a shortening in mean choice reaction time in Session 2, which was sustained in Sessions 3 and 4 consistent with a practice effect. This is a study with well-defined exposures and reports of cause and effect (TCE exposure on reaction time); however, no statistics were presented to support the conclusions or the significance of the findings, and the small sample size is a limitation of the study.

#### **D.1.6.2. Muscular Dyscoordination**

Effects on motor dyscoordination resulting from TCE exposure have been reported in the literature. These impacts are subjective, but may provide additional evidence that TCE can cause adverse psychomotor effects. There are three reports summarized below which suggest that muscular dyscoordination resulted from TCE exposure, although all three have significant limitations due to confounding factors. Rasmussen et al. (1993c) presented findings on muscular dyscoordination as it relates to TCE exposure. This was a historical cohort study conducted on 96 metal degreasers, identified 2 years previously. Subjects were selected from a population of 240 workers from 72 factories in Denmark. Although the papers report a population of 99 participants, tabulated results were presented for a total of only 96. No explanation was provided for this discrepancy. These workers had chronic exposure to fluorocarbon (CFC 113)

*This document is a draft for review purposes only and does not constitute Agency policy.*

( $n = 25$ ) and mostly TCE ( $n = 70$ ; average duration: 7.1 years.). There were no external controls. The range of working full-time degreasing was 1 month to 36 years. Researchers collected data regarding the workers' occupational history, blood and urine tests, as well as biological monitoring for TCE and TCE metabolites. A chronic exposure index (CEI) was calculated based on number of hours per week worked with solvents multiplied by years of exposure multiplied by 45 weeks per year. No TCE air concentrations were reported. Participants were categorized into three groups: (1) "Low exposure:"  $n = 19$ , average full-time exposure = 0.5 years. (2) "Medium exposure:"  $n = 36$ , average full-time exposure = 2.1 years. (3) "High exposure:"  $n = 41$ , average full-time exposure = 11 years. The mean TCA level in the "high" exposure group was 7.7 mg/L (max = 26.1 mg/L). Time-weighted average (TWA) measurements of CFC 113 levels were 260–420 ppm (U.S. and Danish TLV was 500 ppm). A significant trend of dyscoordination from low to high solvent exposure was observed ( $p = 0.003$ ). This study provides evidence of causality for muscular dyscoordination resulting from exposure to TCE, but no measured exposure data were reported.

Additional evidence of the psychomotor effects caused by exposure to TCE are presented in Gash et al. (2007) and Troster and Ruff (1990). There are, however, significant limitations with each of these studies. In Gash et al. (2007), the researchers evaluated the clinical features of 1 Parkinson's disease (PD) patient, identified in a Phase 1 clinical trial study, index case, and an additional 29 coworkers of the patient, all with chronic occupational exposures to TCE. An additional 2 subjects with Parkinson's Disease were included, making the total of 3 Parkinson's disease patients, and 27 non-Parkinson's coworkers making up the study population. Coworkers for the study were identified using a mailed questionnaire to 134 former coworkers. No details are provided in the paper on selection criteria for the 134 former coworkers. Of the 134 former workers sent questionnaires, 65 responded. Twenty-one self-reported no symptoms, 23 endorsed 1–2 symptoms, and 21 endorsed 3 or more signs of Parkinsonism. Fourteen of the 21 with 3 or more signs and 13 of the 21 without any signs agreed to a clinical exam; this group comprises the 27 additional workers examined for Parkinsonian symptoms. No details were provided on nonresponders. All subjects were involved in degreasing with long-term chronic exposure to TCE through inhalation and dermal exposure (14 symptomatic: age range = 31–66, duration of employment range: 11–35 yrs) (13 asymptomatic: age range = 46–63, duration of employment range: 8–33 yrs). The data were compared between groups and with data from 110 age- matched controls. Exposure to TCE is self-reported and based on job proximity to degreasing operations. The paper lacks any description of degreasing processes including TCE usage and quantity. Mapping of work areas indicated that workers with PD worked next to the TCE container, and all symptomatic workers worked close to the TCE container. Subjects underwent a general physical exam, neurological exam and Unified Parkinson's Disease Rating Scale (UPDRS),

*This document is a draft for review purposes only and does not constitute Agency policy.*

timed motor tests, occupational history survey, and mitochondrial neurotoxicity. ANOVA analysis was conducted, comparing symptomatic versus nonsymptomatic workers, and comparing symptomatic workers to age-matched nonexposed controls. No description of the control population ( $n = 110$ ), nor how data were obtained for this group, was presented. The symptomatic non-Parkinson's group was significantly slower in fine motor hand movements than age-matched nonsymptomatic group ( $p < 0.001$ ). The symptomatic group was significantly slower ( $p < 0.0001$ ) than age-matched unexposed controls as measured in fine motor hand movements on the Movement Analysis Panel. All symptomatic workers had positive responses to 1 or more questions on UPDRS Part II (diminished activities of daily life), and/or deterioration of motor functions on Part III. The fine motor hand movement times of the asymptomatic TCE-exposed group were significantly slower ( $p < 0.0001$ ) than age-matched nonexposed controls. Also, in TCE-exposed individuals, the asymptomatic group's fine motor hand movements were slightly faster ( $p < 0.01$ ) than those of the symptomatic group. One symptomatic worker had been tested 1 year prior and his UPDRS score had progressed from 9 to 23. Exposures are based on self-reported information, and no information on the control group is presented. One of the PD patients predeceased the study and had a family history of PD.

Troster and Ruff (1990) reported a case study conducted on two occupationally exposed workers to TCE. Patients were exposed to low levels of TCE. There were 2 groups of  $n = 30$  matched controls (all age and education matched) whose results were compared to the performance of the exposed subjects. Exposure was described as "Unknown amount of TCE for 8 months." Assessment consisted of the San Diego Neuropsychological Test Battery (SDNTB) and "1 or more of" Thematoc Apperception Test (TAT), Minnesota Multiphasic Personal Inventory (MMPI), and Rorschach. Medical examinations were conducted, including neurological, CT scan, and/or chemo-pathological tests, and occupational history was taken, but not described. There were no statistical results reported. Results were reported for each test, but no tests of significance were included, therefore, the authors presented their conclusions for each "case" in qualitative terms, as such: Case 1: Intelligence "deemed" to drop from premorbid function at 1 year 10 months after exposure. Impaired functions improved for all but reading comprehension, visuospatial learning and categorization (abstraction). Case 2: Mild deficits in motor speed, but symptoms subsided after removal from exposure.

#### **D.1.7. Summary Tables**

The following Tables (D-1 through D-3) provide a detailed summary of all the neurological studies conducted with TCE in humans. Tables D-1 and D-2 summarize each individual human study where there was TCE exposure. Table D-1 consists of studies where

humans were primarily or solely exposed to TCE. Table D-2 contains human studies where there was a mixed solvent exposure and TCE was one of the solvents in the mixture. For each study summary, the study population, exposure assessment, methods, statistics, and results are provided. Table D-3 indicates the neurological domains that were tested from selected references (primarily from Table D-1).



**Table D-1. Epidemiological studies: Neurological effects of trichloroethylene**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results																								
Barret et al., 1984	<p>188 workers exposed to TCE occupationally from small and large factories in France (type of factories not disclosed); average age = 41; 6 yrs average exposure time.</p> <p>The workers were divided into high and low exposure groups for both TCE and urinary TCA. No control group was mentioned.</p>	<p>Review of medical records and analysis of TCE atmospheric levels (detector tubes) and level of urinary metabolites measurement (TCA). TCE exposure groups included high exposure group (&gt;150 ppm; <math>n = 54</math>) and low exposure group (&lt;150 ppm; <math>n = 134</math>). Personal factors including age, tobacco use, and alcohol intake were also analyzed; Exposure duration = 7 h/d for 7 yrs; no mention was made regarding whether or not the examiners were blind to the subjects' exposure status.</p>	<p>Complete physical examination including testing visual performance (acuity and color perception), evoked trigeminal potential latencies and audiometry, facial sensitivity, reflexes, and motoricity of the masseter muscles.</p>	<p><math>\chi^2</math> examined distribution of the different groups for comparing high and low exposed workers, one way analysis of variance, Mann Whitney U and t-test for analyzing personal factors.</p>	<p>Symptoms for which TCE role is statistically significant include the following: Trigeminal nerve impairment was reported in 22.2% (<math>n = 12</math>) of workers in the high-exposure group for TCE, 7.4% (<math>n = 10</math>) in the low-exposure group for TCE, 24.4% (<math>n = 10</math>) in the high-exposure group for TCA and 8.2% (<math>n = 12</math>) in the low-exposure group for TCA.</p> <table border="1"> <thead> <tr> <th>TCE Results</th> <th>High dose%</th> <th>Low dose%</th> <th><math>p</math></th> </tr> </thead> <tbody> <tr> <td>Trigeminal nerve impairment</td> <td>22.2</td> <td>7.4</td> <td>&lt;0.01</td> </tr> <tr> <td>Optic nerve impairment</td> <td>18.5</td> <td>4.5</td> <td>&lt;0.01</td> </tr> <tr> <td>Headache</td> <td>14.8</td> <td>0.75</td> <td>&lt;0.001</td> </tr> <tr> <td>Dizziness</td> <td>20.3</td> <td>19.4</td> <td>NS</td> </tr> <tr> <td></td> <td>13</td> <td>4.5</td> <td><math>0.05 &lt; p &lt; 0.06</math></td> </tr> </tbody> </table> <p>Symptoms for which TCE role is possible, but not statistically significant = deafness, nystagmus, GI symptoms, morning cough, change in tumor, eczema, palpitations, conjunctivitis. Symptoms for which there is a synergistic toxic role for TCE and alcohol (<math>p &lt; 0.05</math>) = liver impairment and degreaser flush. Trigeminal sensory evoked potentials are suggested as a good screening test.</p>	TCE Results	High dose%	Low dose%	$p$	Trigeminal nerve impairment	22.2	7.4	<0.01	Optic nerve impairment	18.5	4.5	<0.01	Headache	14.8	0.75	<0.001	Dizziness	20.3	19.4	NS		13	4.5	$0.05 < p < 0.06$
TCE Results	High dose%	Low dose%	$p$																										
Trigeminal nerve impairment	22.2	7.4	<0.01																										
Optic nerve impairment	18.5	4.5	<0.01																										
Headache	14.8	0.75	<0.001																										
Dizziness	20.3	19.4	NS																										
	13	4.5	$0.05 < p < 0.06$																										

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Barret et al., 1987	104 occupationally exposed workers highly exposed to TCE during work as degreaser machine operators in France. Controls: 52 healthy, nonexposed controls of various ages who were free from neurological problems.	Urinary analysis determined TCE and TCA rates. The average of the last 5 measurements were considered indicative of the average level of past exposure. Mean exposure 8.2 yrs, average daily exposure 7 hrs/d. Mean age 41.6 yrs.	Evoked trigeminal potentials were studied while eyes closed and fully relaxed. Also, physical exams with emphasis on nervous system, a clinical study of facial sensitivity, and of the reflexes depending on the trigeminal nerve were systematically performed. Normal latency and amplitude values for TSEP obtained from data from control population. Normal response characterized from 4 main peaks, alternating from negative to positive, respective latency of 12.8 ms (SD = 0.6), 19.5 ms (SD = 1.3), 27.6 ms (SD = 1.6), and 36.8 ms (SD = 2.2), mean amplitude of response is 2.5 $\mu$ v (SD = 0.5 $\mu$ v). Pathological responses were results 2 ½ SDs over the normal value.	Student's t-test and one-way ANOVA used as well as nonparametric tests Mann-Whitney U test and Kruskal-Wallis test. Also decision matrix and the analysis of the receiver operating curve to appreciate the accuracy of the TSEP method. The distribution of the different populations was compared by a chi square test.	Dizziness (71.4%), headache (55.1%), asthenia (46.9%), insomnia (24.4%), mood perturbation (20.4%), and sexual problems (12.2%) were found. Symptomatic patients had significantly longer exposure periods and were older than asymptomatic patients. 17.3% of patients had trigeminal nerve symptoms. Bilateral hypoesthesia with reflex alterations in 9 cases. Hypoesthesia was global and predominant in the mandibular and maxillary nerve areas. Several reflex abolitions were found without facial palsy and without convincing hypoesthesia in 9 cases. Corneal reflexes were bilaterally abolished in 5 cases as were naso-palpebral reflexes in 6 cases; length of exposure positively correlated with functional manifestations ( $p < 0.01$ ); correlation between symptoms and exposure levels were nonsignificant; 40 (38.4%) subjects had pathological response to TSEP with increased latencies, amplitude or both; of these 28 had normal clinical trigeminal exam and 12 had abnormal exam; TSEP was positively correlated with length of exposure ( $p < 0.01$ ); and with age ( $p < 0.05$ ), but not with exposure concentration; trigeminal nerve symptoms ( $n = 18$ ) were positively correlated with older age ( $p < 0.001$ ).

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Barret et al., 1982	Eleven workers with chronic TCE exposure; 9 were suffering effects of solvent intoxication; 2 were work place controls. Control group was 20 unexposed subjects of all ages.	Selected following clinical evaluations of their facial sensitivity and trigeminal nerve reflexes; exposures verified by urinalysis. Presence of TCE and TCA found. (Exposure rates not reported).	Somatosensory evoked potential (SEP) following stimulation of the trigeminal nerve through the lip alternating right and left by a bipolar surface electrode utilizing voltage, usually 75 to 80 V, just below what is necessary to stimulate the orbicularis oris muscle. Duration was approx. 0.05 ms stimulated 500 times (2×/sec).	SEP recordings illustrated from trigeminal nerve graphs.	3 pathological abnormalities present in exposed (TCE intoxicated) workers: (1) in 8 workers higher voltage required to obtain normal response, (2) excessive delay in response observed twice, (3) excessive graph amplitude noted in 3 cases. One subject exhibited all 3 abnormalities. Correlation was reported between clinical observation and test results. Most severe SEP alternations observed in subjects with the longest exposure to TCE (although exposure levels or exposure durations are not reported). No statistics presented.
Burg et al., 1995	From an NHIS TCE subregistry of 4,281 (4,041 living and 240 deceased) residents environmentally exposed to TCE via well water in Indiana, Illinois, and Michigan; compared to NHIS registrants.	Morbidity baseline data were examined from the TCE Subregistry from the NER developed by the ATSDR; were interviewed in the NHIS.	Self report via face-to-face interviews—25 questions about health conditions; were compared to data from the entire NHIS population; neurological endpoints were hearing and speech impairments.	Poisson Regression analysis model used for registrants 19 and older. Maximum likelihood estimation and likelihood ratio statistics and Wald CI; TCE subregistry population was compared to larger NHIS registry population.	Speech impairments showed statistically significant variability in age-specific risk ratios with increased reporting for children $\leq 9$ yrs (RR: 2.45, 99% CI: 1.31, 4.58) and for registrants $\geq 35$ yrs (data broken down by 10-yr ranges). Analyses suggest a statistically significant increase in reported hearing impairments for children $\leq 9$ yrs (RR: 2.13, 99% CI: 1.12, 4.06). It was lower for children 10–17 yrs (RR: 1.12, 99% CI: 0.52, 2.44) and $\leq 0.32$ for all other age groups.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Burg and Gist, 1999	4,041 living members of the National Exposure Registry's Trichloroethylene Subregistry; 97% white; mean age 34 yrs (SD = 19.9 yrs.); divided in 4 groups based on type and duration of exposure; analysis reported only for 3,915 white registrants; lowest quartile used as control group.	All registrants exposed to TCE through domestic use of contaminated well water; 4 exposure Subgroups, each divided into quartiles: (1) Maximum TCE measured in well water, exposure subgroups: 2–12 ppb; 12–60 ppb; 60–800 ppb; (2) Cumulative TCE exposure subgroups: <50 ppb, 50–500 ppb, 500–5,000 ppb, >5,000 ppb; (3) Cumulative chemical exposure subgroups: include TCA, DCE, DCA, in conjunction with TCE, with the same exposure Categories as in # 2; (4) Duration of exposure subgroups: <2 yrs, 2–5 yrs, 5–10 yrs., >10 yrs.; 2,867 had TCE exposure of ≤50 ppb; 870 had TCE exposure of 51–500 ppb; 190 had TCE exposure of 501–5,000 ppb; 35 had TCE exposure >5,000 ppb.	Interviews (occupational, environmental, demographic, and health information); A large number of health outcomes analyzed, including speech impairment and hearing impairment.	Logistic Regression, Odds Ratios; lowest quartile used as reference population.	When the registrants were grouped by duration of exposure to TCE, a statistically significant association (adjusted for age and sex) between duration of exposure and reported hearing impairment was found. The prevalence odds ratios were 2.32 (95% CI: 1.18, 4.56) (>2 to <5 yrs); 1.17 (95% CI: 0.55, 2.49) (>5 to <10 yrs); and 2.46 (95% CI: 1.30, 5.02) (>10 yrs); Higher rates of speech impairment (not statistically significant) associated with maximum and cumulative TCE exposure, and duration of exposure.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Buxton and Hayward, 1967	This was a case study on 4 workers exposed to very high concentrations of TCE, which resulted from an industrial accident. No controls were evaluated.	Case 1 was a 44-yr old man exposed for 10 min; Case 2 was a 39-yr old man exposed for 30 min; Case 3 was a 43-yr old man exposed for 2.5 h; Case 4 was a 39-yr old man exposed for 4 h. TCE concentrations were not reported.	Clinical evaluations were conducted by a physician when patients presented with symptoms; numbness of face, ocular pain, enlarged right blind spot, nausea, loss of taste, headache, dizziness, unsteadiness, facial diplesia, loss of gag and swallowing reflex, absence of corneal reflex, and reduction of trigeminal response.	There was no statistical assessment of results presented.	Case 1 exhibited headaches and nausea for 48 h, but had a full recovery. Case 2 exhibited nausea and numbness of face, but had a full recovery. Case 3 was seen and treated at a hospital with numbness of face, insensitivity to pin prick over the trigeminal distribution, ocular pain, enlarged right blind spot, nausea, and loss of taste. No loss of mental faculty was observed. Case 4 was seen and treated for headache, nausea, dizziness, unsteadiness, facial diplesia, loss of gag and swallowing reflex, facial analgesia, absence of corneal reflex, and reduction of trigeminal response. The patient died and was examined postmortem. There was demyelination of the 5 <sup>th</sup> cranial nerve evident.
Chalupa et al., 1960	This was a case study conducted on 22 patients with acute poisoning caused by carbon monoxide and industrial solvents. Six subjects were exposed to TCE (doses not known). Average age 38.	No exposure data were reported.	Medical and psychological exams were given to all subjects. These included EEGs, measuring middle voltage theta activity of 5–6 sec duration. Subjects were tested for memory disturbances.	No statistics were performed.	80% of those with pathological EEG displayed memory loss; 30% of those with normal EEGs displayed memory loss. Pathology and memory loss were most pronounced in subjects exposed to carbon monoxide.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
El Ghawabi et al., 1973	30 money printing shop workers occupationally exposed to TCE; Controls: 20 age and SES matched nonexposed males and 10 control workers not exposed to TCE but exposed to inks used in printing.	Air samples on 30 workers. Mean TCE air concentrations ranged from 41 to 163 ppm throughout the Intalgio process. Colorimetric determination of both TCA and total trichloro-compounds in urine with Fujiware reaction.	Inquiries about occupational, past and present medical histories, and family histories in addition to age and smoking habits. EKGs were performed on 25 of the workers. Lab investigations included complete blood and urine analysis, and routine liver function tests.	Descriptive statistics and central tendency evaluation for metabolites; no stats reported for neurological symptoms.	Most frequent symptoms: prenarcoptic headache (86% vs. 30% for controls), dizziness (67% vs. 6.7% for controls), and sleepiness (53% vs. 6% for controls) main presenting symptoms in addition to suppression of libido. Trigeminal nerve involvement was not detected. The concentration of total trichloro-compounds increased toward mid-week and was stationary during the last 2 working days. Metabolites of total trichloroacetic acid and trichloroethanol are only proportional to TCE concentrations up to 100 ppm.
Feldman et al., 1988	21 Massachusetts residents with alleged chronic exposure to TCE in drinking water; 27 laboratory controls.	TCE in residential well water was 30–80 times greater than U.S. EPA MCL; maximum reported concentration was 267 ppb; other solvents also present.	BR used as an objective indicator of neurotoxic effects of TCE; clinical neurological exam, EMGs to evaluate blink reflex, nerve conduction studies, and extensive neuropsychological testing.	Student's t-test used for testing the difference between the group means for the Blink reflex component latencies.	Highly significant differences in the conduction latency means of the BR components for the TCE exposed population vs. control population, when comparing means for the right and left side R1 to the controls ( $p < 0.001$ ). The mean R1 BR component latency for the exposed group was 11.35 ms, SD = 0.74 ms, 95% CI: 11.03–11.66. The mean for the controls was 10.21 ms, SD = 0.78 ms, 95% CI: 9.92–10.51; $p < 0.001$ . Suggests a subclinical alteration of the trigeminal nerve function due to chronic, environmental exposure to TCE.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Feldman et al., 1992	18 workers occupationally exposed to TCE; 30 laboratory controls.	Reviewed exposure histories of each worker (job type, length of work) and audited medical records to categorize into three exposure categories: “extensive,” “occasional,” and “chemical other than TCE”.	Blink reflexes using TECA 4 EMG.	Non-Gaussian distribution and high coefficient of variance data were log-transformed and then compared to the log-transformed control mean values. MRV was calculated by subtracting the subjects value ( $x$ ) from the control group mean ( $M$ ), and the difference is divided by the control group standard deviation.	The “extensive” group revealed latencies greater than 3 SD above the nonexposed group mean on R1 component of blink reflex; none of the “occasional” group exhibited such latencies, however, two of them demonstrated evidence of demyelinating neuropathy on conduction velocity studies; the sensitivity, or the ability of a positive blink reflex test to correctly identify those who had TCE exposure, was 50%. However, the specificity was 90%, which means that of those workers with no exposure to TCE, 90% demonstrated a normal K1 latency. Subclinical alteration of the Vth cranial nerve due to chronic occupational exposure to TCE is suggested.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Gash et al., 2007	30 Parkinson's Disease patients and 27 non-Parkinson coworkers exposed to TCE; No unexposed controls.	Mapping of work areas.	General physical exam, neurological exam and UPDRS, timed motor tests, and occupational history survey; mitochondrial neurotoxicity; Questionnaire mailed to 134 former non-Parkinson's workers, (14 symptomatic of parkinsonism: age range = 31–66, duration of employment range: 11–35 yrs) (13 asymptomatic: age range = 46–63, duration of employment range: 8–33 yrs);.	Workers' raw scores given; ANOVA comparing symptomatic vs. nonsymptomatic workers.	Symptomatic non-Parkinson's group was significantly slower in fine motor hand movements than age-matched nonsymptomatic group ( $p < 0.001$ ); All symptomatic workers had positive responses to 1 or more questions on UPDRS Part I and Part II, and/or had signs of parkinsonism on Part III; One symptomatic worker had been tested 1 yr. prior and his UPDRS score had progressed from 9 to 23.



**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Grandjean et al., 1955	80 workers employed in 10 different factories of the Swiss mechanical engineering industry exposed to TCE, seven of whom stopped working with TCE from 3 wks to 6 yrs prior; no unexposed control group.	Vapors were collected in ethylic alcohol 95%. Volume of air was checked using a flowmeter, and quantitatively measured according to the method of Truhaut (1951), which is based on a colored reaction between TCE and the pyridine in an alkaline medium (with modifications). Urine analysis of TCA levels; TCE air concentrations varied from 6–1,120 ppm depending on time of day and proximity to tanks, but mainly averaged between 20–40 ppm. Urinalysis varied from 30 mg/L to 300 mg/L; Could not establish a relationship between TCE eliminated through urine and TCE air levels. Four exposure groups estimated based on air sampling data.	Medical exam, including histories; Blood and biochem. tests, and psychiatric exam. Psychological exam; Meggendorf, Bourdon, Rorschach, Jung, Knoepfel's "thirteen mistakes" test, and Bleuler's test.	Coefficient of determination, Regression coefficient.	Men working all day with TCE showed on average larger amounts of TCA than those who worked part time with TCE. Relatively high frequency of subjective complaints, of alterations of the vegetative nervous system, and of neurological and psychiatric symptoms. 34% had slight or moderate psycho-organic syndrome; 28% had neurological changes; There is a relationship between the frequency of those alterations and the degree of exposure to TCE. There were significant differences ( $p = 0.05$ ) in the incidence of neurological disorder between Groups I and III, while between Groups II and III there were significant differences ( $p = 0.05$ ) in vegetative and neurological disorders. Based on TCA eliminated in the urine, results show that subjective, vegetative, and neurological disorders were more frequent in Groups II and III than in Group I. Statistical analysis revealed the following significant differences ( $p < 0.01$ ): subjective disorders between I and II ; vegetative disorders between I and II and between I and III; neurological disorders between I and (II and III). Vegetative, neurological, and psychological symptoms increased with the length of exposure to TCE. The following definite differences were shown by statistical analysis ( $p < 0.03$ ) : vegetative disorders between I and IV ; neurological disorders between I and II and between I and IV; psychological disorders between I and III and between I and IV.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

<b>Reference</b>	<b>Study population</b>	<b>Exposure assessment and biomarkers</b>	<b>Tests used</b>	<b>Statistics</b>	<b>Results</b>
Gun, et al., 1978	8 exposed: 4 female workers from one plant exposed to TCE and 4 female workers from another plant exposed to TCE + nonhalogenated hydrocarbon solvent used in degreasing; control group ( $n = 8$ ) consisted of 4 female workers from each plant who did not work near TCE.	Air sampled continuously over separate 10 min durations drawn into a Davis Halide Meter. Readings taken every 30 sec.; ranged from 3–419 ppm.	Eight-Choice reaction times carried out in four sessions; 40 reaction time trials completed.	Variations in RT by level of exposure; ambient air exposure TCE concentrations and mean air TCE values.	TCE only group had consistently high mean ambient air TCE levels (which exceeded the 1978 TLV of 100 ppm) and showed a mean increase in reaction time, with a probable cumulative effect. In TCE + solvent group, ambient TCE was lower (did not exceed 100 ppm) and mean reaction time shortened in Session 2, then rose subsequently to be greater than at the start. Both control groups showed a shortening in mean choice reaction time in Session 2 which was sustained in Sessions 3 and 4 consistent with a practice effect; No stats provided.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Hirsch et al., 1996	106 residents of Roscoe, a community in Illinois on the Rock River, in direct proximity to an industrial plant that released an unknown amount of TCE into the River. All involved in litigation. Case series report; No unexposed controls.	Random testing of the wells between 1983–84 revealed groundwater in wells to have levels of TCE between 0 to 2,441 ppb; distance of residence from well used to estimate exposure level.	Medical, neurologic, and psychiatric exams and histories. For those who complained of headaches, a detailed headache history was taken, and an extensive exam of nerve-threshold measurements of toes, fingers, face, olfactory threshold tests for phenylethyl methylethyl carbinol, brain map, Fast Fourier Transform (FFT), P300 Cognitive auditory evoked response, EEG, Visual Evoked Response (VER), Somato sensory Evoked Potential (SSER), Brainstem Auditory Evoked Response (BAER), MMPI-II, MCMI-II, and Beck Depression Inventory were also given.	Student t-test, Chi square analysis, nonparametric t-test and ANOVA, correlating all history, physical exam findings, test data, TCE levels in wells, and distance from plant.	66 subjects (62%) complained of headaches, Diagnosis of TCE-induced cephalgia was considered credible for 57 patients (54%). Retrospective TCE level of well water or well's distance from the industrial site analysis did not correlate with the occurrence of possibly-TCE induced headaches. Studies that were not statistically significant with regard to possible TCE-cephalalgia included P300, FFT, VER, BAER, MMPI, MCMI, Beck Depression Inventory, SSER, and nerve threshold measurements. Headache might be associated with exposure to TCE at lower levels than previously reported. Headaches mainly occurred without sex predominance, gradual onset, bifrontal, throbbing, without associated features; No quantitative data presented to support statement of headache in relation to TCE exposure levels, except for incidences of headache reporting and measured TCE levels in wells.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn and Thornton, 1996	Group A: Randomly selected registered voters from Arizona and Louisiana with no exposure to TCE: <i>n</i> = 264 unexposed volunteers aged 18–83; Group B volunteers from California <i>n</i> = 29 17 males and 12 females to validate the equations; Group C exposed to TCE and other chemicals residentially for 5 yrs or more <i>n</i> = 237.	No exposure or groundwater analyses reported.	Reaction speed using a timed computer visual-stimulus generator; Compared groups to plotted measured SRT and CRT Questionnaire to eliminate those exposed to possibly confounding chemicals.	Box-Cox transformation for dependent and independent variables. Evaluated graphical methods to study residual plots. Cooks distance statistic measured influence of outliers examined. Lack-of-fit test performed on Final model and F statistic to compare estimated error to lack-of-fit component of the model’s residual sum of squared error. Final models were validated using Group B data and paired t-test to compare observed values for SRT and CRT. F statistic to test hypothesis that parameter estimates obtained with Group B were equal to those of the model.	Group A: SRT = 282 ms CRT = 532 ms Group B: SRT = 269 ms CRT = 531 ms Group C: SRT = 334 ms CRT = 619 ms $Lg(SRT) = 5.620$ , $SD = 0.198$ Regression equation for $Lg(CRT) = 6.094389 + 0.0037964 \times \text{age}$ . TCE exposure produced a step increase in SRT and CRT, but no divergent lines. Coefficients from Group A were valid for Group B. Predicted value for SRT and for CRT, plus 1.5 SDs. selected 8% of the model group as abnormal.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn and Warshaw, 1993	Well-water exposed subjects to 6 to 500 ppb of TCE for 1 to 25 yrs; 544 recruited test subjects; Group 1 = 196 exposed family members of subjects with cancer or birth defects; Group 2 = 178 from exposed families without cancer or birth defects; Group 3 = 170 exposed parents whose children had birth defects and rheumatic disorders; Controls: 68 referents and 113 histology technicians (HTs) without environmental exposure to TCE.	Well-water was measured from 1957 to 1981 by several governmental agencies, and average annual TCE exposures were calculated and then multiplied by each individual's years of residence for 170 subjects.	Neurobehavioral testing - augmented NBT; Eye Closure and Blink using EMG; neuropsychological (NPS) test - Portions of Wechsler's Memory Scale, and WAIS and embedded figures test, grooved pegboard, Trail Making A and B, POMS, and Culture Fair Test; neurophysiological (NPH) testing - Simple visual reaction time, body balance apparatus, cerebellar function, proprioception, visual, associative links and motor effector function.	Two sided student t-test with a $p < 0.05$ .  Linear regression coefficients to test how demographic variables or other factors may contribute.	Exposed subjects had lower intelligence scores and more mood disorders.  NPH: Significant impairments in sway speed with eyes open and closed, blink reflex latency (R-1), eye closure speed, and two choice visual reaction time.  NPS: Significant impairments in Culture Fair (intelligence) scores, recall of stories, visual recall, digit span, block design, recognition of fingertip numbers, grooved pegboard, and Trail Making A and B.  POMS: all subtests, but the fatigue, were elevated. Mean speeds of sway were greater with eyes open at $p < 0.0001$ and with eyes closed $p < 0.05$ in the exposed group compared to the combined referents. The exposed group mean simple reaction time was 67 msec longer than the referent group $p < 0.0001$ . Choice reaction time (CRT) of the exposed subjects was 93 msec longer in the third trial ( $p < 0.0001$ ) than referents. It was also longer in all trials, and remained significantly different after age adjustment. Eye closure latency was slower for both eyes in the exposed and significantly different ( $p < 0.0014$ ) on the right compared to the HT referent group.
Kilburn, 2002b	236 residents chronically exposed to TCE and associated solvents, including DCE, PCE, and vinyl chloride, in the environment from a	Exposure estimate based on groundwater plume based on contour mapping; concentrations between 0.2–10,000 ppb of TCE over a 64 km <sup>2</sup> area; additional	Simple reaction time, choice reaction time, Balance sway speed (with eyes open and eyes closed), color errors, blink reflex latency, Supra orbital	Descriptive statistics; ANCOVA; step-wise adjustment of demographics.	The principal comparison, that was between the 236 exposed persons and the 161 unexposed regional controls, revealed 13 significant differences ( $p < 0.05$ ). SRTs and CRTs were delayed. Balance was abnormal with excessive sway speed (eyes closed), but this was not true when both eyes were open. Color discrimination

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn, 2002b (continued)	<p>nearby microchip plant, some involved in litigation, prior to 1983 and those who lived in the area between 1983 and 1993 during which time dumping of chlorinated solvents had supposedly ceased and clean-up activities had been enacted; Controls: 67 referents from northeast Phoenix, who had never resided near the 2 plants (mean distance = 2,000 m, range = 1,400–3,600 m from plants) and 161 regional referents from Wickenburg, AZ up-wind of Phoenix, recruited via random calls made to numbers on voter registration rolls, matched to exposed subjects by age and years of education, records showed no current or past water contamination in the areas.</p>	<p>associated solvents, including DCE, PCE, and vinyl chloride, No air sampling.</p>	<p>tap (left and right), Culture Fair A, Vocabulary, Pegboard, Trail Making A and B, Immediate verbal recall, POMS; Pulmonary Function; The same examiners who were blinded to the subjects' exposure status examined the Phoenix group, but the Wickenburg referents' status was known to the examiners. Exact order or timing of testing not stated.</p>		<p>errors were increased. Both right and left blink reflex latencies (R-1) were prolonged. Scores on Culture Fair 2A, vocabulary, grooved pegboard (dominant hand), trail making A and B, and verbal recall (i.e., memory) were decreased in the exposed subjects. Litigation is suggested but not stated and study paid by lawyers. Litigation status may introduce a bias, particularly if no validity tests were used.</p>

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

<b>Reference</b>	<b>Study population</b>	<b>Exposure assessment and biomarkers</b>	<b>Tests used</b>	<b>Statistics</b>	<b>Results</b>
Kilburn, 2002b	236 residents exposed environmentally from a nearby microchip plant (exact number of litigants not stated); 156 individuals exposed for >10 y compared to 80 individuals <10 y of exposure; Controls: 58 nonclaimants in 3 areas within exposure zone (Zones A, B, and C).	No discussion of exposure assessment methods and results. Solvents included TCE, DCE, PCE, and vinyl chloride; concluded exposure is primarily due to groundwater plume rather than air releases.	Simple reaction time, choice reaction time, Balance sway speed (with eyes open and eyes closed), color errors, blink reflex latency, Supra orbital tap (left and right), Culture Fair A, Vocabulary, Pegboard, Trail Making A and B, Immediate verbal recall, POMS.	Descriptive statistics, Regression analysis; Similar study to the one reported above with the exception of looking at the effects of duration of residence, proximity to the microchip plant, and being involved in litigation.	Insignificant effects of longer duration of residence. No effect of proximity and litigation. Effects of longer duration of residence modest and insignificant. No effect of proximity. No litigation effect. Zone A- 100 clients were not different from the 9 nonclients. Zone B, nonclients were more abnormal in color different than clients and right-sided blink was less abnormal in nonclients. Zone C, 9 of the 13 measurements were not significantly different. 26 of the original 236 subjects re-tested in 1999: maintained impaired levels of functioning and mood; No tests of effort and malingering used, limiting interpretations. Again, no tests of effort and malingering were used, thus, limiting interpretation. Litigation is suggested but not stated and study paid by lawyers. Litigation status may introduce a bias, particularly if no validity tests were used.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Landrigan et al., 1987	13 Pennsylvania residents exposed through drinking and bathing water contaminated by approximately 1,900 gallon TCE spill; Feb 1980: 9 workers exposed to TCE while degreasing metal in pipe manufacturing plant and 9 unexposed controls (mean ages were 42.7 exposed and 46.4-y old unexposed; mean durations of employment = 4.4, exposed, and 9.4 y, unexposed.; May 1980: 10 exposed workers and same 9 unexposed worker controls from Feb monitoring.	<p>Community Evaluation: Nov 1979- Questionnaires on TCE and other chemical exposures, and occurrence of signs and symptoms of exposure to TCE, morning urine samples, urine samples analyzed colorimetrically for total trichloro-compounds.</p> <p>Occupational Evaluations (In workers): breathing-zone air samples( mean 205 mg/m<sup>3</sup>; 37 ppm); medical evaluations, pre and post shift spot urine samples in Feb and again in May, mid and post shift venous blood samples during the May survey,</p>	Community evaluation, occupational evaluations; urine evaluations for TCE metabolites; Questionnaires to evaluate neurologic effects and symptoms; ISO concentrations, Map of TCE in groundwater.	Descriptive statistics	<p>Community Evaluation: No urinary TCA detected in community population except for 1 resident also working at plant and 1 resident with no exposure; Occupational Evaluation: Range 117–357 mg/m<sup>3</sup> – (21–64 ppm). Feb: airborne exposures exceeded NIOSH limit by up to 222 mg/m<sup>3</sup> (40 ppm)(NIOSH TWA &lt;135 mg/m<sup>3</sup>). (24 ppm). Short term exposure exceeded NIOSH values of 535 mg/m<sup>3</sup> (96 ppm) by up to 1,465 mg/m<sup>3</sup> (264 ppm). Personal breathing zone of other workers within recommended limits (0.5–125 mg/m<sup>3</sup>) (0.1–23 ppm). 7 exposed workers reported acute symptoms, including fatigue, light-headedness, sleepiness, nausea, headache, consistent with TCE exposure; No control workers reported such symptoms; Prevalence of 1 or more symptoms 78% in exposed worker group, 0% in control worker group; Symptoms decreased after recommendations were in place for 3 mos (may testing) for reduced exposures.</p>



Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Liu et al., 1988	103 workers from factories in Northern China, exposed to TCE (79 men, 24 women), during vapor degreasing production or operation. The unexposed control group included 85 men and 26 women.	Exposed to TCE, mostly at less than 50 ppm; concentration of breathing zone air during entire shift measured by diffusive samplers placed on the chest of each worker; divided into three exposure groups; 1–10 ppm, 11–50 ppm and 51–100 ppm; Also, hematology, serum biochemistry, sugar, protein, and occult blood in urine were collected.	Self-reported subjective symptom questionnaire.	Prevalence of affirmative answers = total number of affirmative answers divided by (number of respondents × number of questions); $\chi^2$ .	Dose-response relationship established in symptoms such as nausea, drunken feeling, light-headedness, floating sensation, heavy feeling of the head, forgetfulness, tremors and/or cramps in extremities, body weight loss, changes in perspiration pattern, joint pain, and dry mouth (all $\geq 3$ times more common in exposed workers); “bloody strawberry jam-like feces” was borderline significant in the exposed group and “frequent flatus” was statistically significant. Exposure ranged up to 100 ppm, however, most workers were exposed below 10 ppm, and some at 11–50 ppm. Contrary to expectations, production plant men had significantly higher levels of exposure (24 had levels of 1–10 ppm, 15 had levels of 11–50 ppm, 4 had levels of 51–100 ppm) than degreasing plant men (31 had levels of 1–10 ppm, 2 had levels of 11–50 ppm, 0 had levels of 51–100 ppm); $p < 0.05$ by chi-square test. No significant difference ( $p > 0.10$ ) was found in women workers. The differences in exposure intensity between men and women was of borderline significance ( $0.05 < p < 0.10$ ).

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
McCunney, 1988	This is a case study conducted on 3 young white male workers exposed to TCE in degreasing operations. There were no controls included. Case 1 was a 25-yr old male, Case 2 was a 28-yr old white male, Case 3 was a 45-yr old white male.	Case 1: TCE in air at the work place was measured at 25 ppm, but his TCA in urine was measured at 210 mg/L. This is likely due to dermal exposure while cleaning metal rods in TCE. Case 2: no TCE exposure data presented, TCA at 9 mg/L after 6 mos; Case 3: no TCE exposure data presented.	Clinical evaluation of loss of balance, light headedness, resting tremor, blurred vision, and dysdiadochokinesia, change in demeanor and loss of coordination, cognitive changes were noted, as well as depression; CT scan, EEG, nerve conductivity, and visual and somatosensory evoked response. Neurological exams included sensitivity to pinprick over the face; Ophthalmic evaluation.	There were no statistical analyses of results presented.	<p>Case 1 was a 25-yr old male, who presented with a loss of balance, light headedness, resting tremor, blurred vision, and dysdiadochokinesia. The subject had been in a car accident and suffered head injuries. He later returned with a change in demeanor and loss of coordination. He showed a normal CT scan, EEG, nerve conductivity, and visual and somatosensory evoked response. Neurological exams revealed reduced sensitivity to pinprick over the face, deep tendon reflexes were reduced, mild to moderate cognitive changes were noted, as well as depression. Ophthalmic evaluation was normal. He was removed from the TCE exposure and appeared to recover.</p> <p>Case 2 was a 28-yr old white male who presented with numbness and shooting pains in fingers. He exhibited anorexia, tiredness. He worked in a degreasing operation for a jeweler using open containers filled with TCE in a small, unventilated room. There were no exposure data provided, but his TCA was 9 mg/L at 6 mos after exposure. He had been hospitalized with hepatitis previously. No neurological tests were administered.</p> <p>Case 3 was a 45-yr old white male who presented with numbness in hands and an inability to sleep. He exhibited slurred speech. He was positive for blood in stool, but had a history of duodenal ulcers.</p>

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Mhiri et al., 2004	23 phosphate industry workers exposed to TCE for 6 h/d for at least 2 yrs while cleaning walls to be painted; Controls: 23 unexposed workers from the department of neurology.	Measurement of urinary metabolites of TCE were performed 3 times/worker. Blood tests and hepatic enzymes were also collected.	Trigeminal somatosensory evoked potentials recorded using Nihon-Kohden EMG- evoked potential system; baseline clinical evaluations regarding facial burn or numbness, visual disturbances, restlessness, concentration difficulty, fatigue, mood changes, assessment of cranial nerves, quality of life; biological tests described under biomarkers.	Paired or unpaired Student's t-test as appropriate. <i>p</i> -value set at <0.05. Spearman rank-correlation procedure was used for correlation analysis.	Abnormal TSEP were observed in 6 workers with clinical evidence of Trigeminal involvement and in 9 asymptomatic workers. A significant positive correlation between duration of exposure and the N2 latency ( <i>p</i> < 0.01) and P2 latency ( <i>p</i> < 0.02) was observed. Only one subject had urinary TCE metabolite levels over tolerated limits. TCE air contents were over tolerated levels, ranging from 50–150 ppm.
Mitchell and Parsons-Smith, 1969	This was a case study of 1 male patient, age 33, occupational exposed to TCE during degreasing. There were no controls.	No exposure data are presented.	Trigeminal nerve, loss of taste, X-rays of the skull, EEG, hemoglobin, and Wassermann reaction.	No statistics provided.	The patient had complete analgesia in the right trigeminal nerve and complete loss of taste, patient complained of loss of sensation on right side of face, and uncomfortable right eye, as well as vertigo and depression. X-rays of the skull, EEG, hemoglobin, and Wassermann reaction were all normal.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Nagaya et al., 1990	84 male workers ages 18–61 (mean 36.2) constantly using TCE in their jobs. Duration of employment (i.e., exposure) 0.1–34.0 yrs, (mean 6.1 yrs; SD = 5.9). Controls: 83 age-matched office workers and students with no exposure.	Workers exposed to about 22-ppm TCE in air. Serum dopamine- $\beta$ -hydroxylase (DBH) activity levels measured from blood. Urinary total trichloro-compounds (U-TTC) also measured.	Blood drawn during working time and DBH activities were analyzed; Spot urine collected at time of blood sampling and U-TTC determined by alkaline-pyridine method.	Student's t-test and linear correlation coefficient. Results of U-TTC presented by age groups: $\leq 25$ ; 26–40; $\geq 41$ .	A slight decrease in serum DBH activity with age was noted in both groups. Significant inverse correlation of DBH activity and age was found in workers ( $r = -0.278$ , $0.01 < p < 0.02$ ), but not in controls ( $r = -0.182$ , $0.05 < p < 0.1$ ). No significant differences between mean serum DBH activity levels by age groups for workers and corresponding controls in any age group. Workers' U-TTC levels: 3.8 to 1,066.4 mg/L (M = 133.6 mg/L); U-TTC not detected in controls. Serum DBH activity levels in workers independent of U-TTC levels and duration of employment. Results suggest that chronic occupational exposure to TCE did not influence sympathetic nerve activity.
Reif et al., 2003	143 residents of the Rocky Mountain Arsenal community of Denver whose water was contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986; Referent group at lowest concentration (<5 ppb).	Hydraulic simulation model used in conjunction with a GIS estimated residential exposures to TCE; Approximately 80% of the sample exposed to TCE exceeding MCL of 5 ppb and approximately 14% exceeded 15 ppb. High exposure group >15 ppb, low exposure referent group <5 ppb, medium exposure group $5 < x < 15$ ppb.	NCTB, tests of visual contrast sensitivity, POMS.	Multivariate Model.	Statistical significance was approached as a result of high TCE exposure vs. referent group; poorer performance on the digit symbol ( $p = 0.07$ ), contrast sensitivity C test ( $p = 0.06$ ), and contrast sensitivity D test ( $p = 0.07$ ), and higher mean scores for depression ( $p = 0.08$ ). Alcohol was an effect modifier in high-exposed individuals—statistically significant on the Benton, digit symbol, digit span, and simple reaction time tests, as well as for confusion, depression, and tension.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen and Sabroe, 1986	368 metal workers working in degreasing at various factories in Denmark (industries not specified) with chlorinated solvents; 94 controls randomly selected semiskilled metal workers from same area; mean age: 37.7 (range: 17–65+). Total 443 men; 19 women.	Questionnaire: categorized in 4 groups; 3 exposure groups plus control: (1) currently working with chlorinated solvents ( $n = 171$ ; average duration: 7.3 yrs, 16.5 h/wk; 57% TCE and 37% 1,1,1-trichloroethane), (2) currently working with other solvents ( $n = 131$ ; petroleum, gasoline, toluene, xylene), (3) previously (1–5 yrs) worked with chlorinated or other solvents ( $n = 66$ ) (4) never worked with organic solvents ( $n = 94$ ).	Questionnaire: 74 items about neuropsychological symptoms (memory, concentration, irritability, alcohol intolerance, sleep disturbance, fatigue).	Chi-square; Odds ratios; t-test; logistic regression.	Neuropsychological symptoms significantly more prevalent in the chlorinated solvents-exposed group; TCE caused the most “inconveniences and symptoms;” dose response between exposure to chlorinated solvents and chronic neuropsychological symptoms (memory [ $p < 0.001$ ], concentration [ $p < 0.02$ ], irritability [ $p < 0.004$ ], alcohol intolerance [ $p < 0.004$ ], forgetfulness [ $p < 0.001$ ], dizziness [ $p < 0.005$ ], and headache [ $p < 0.01$ ]); Significant associations between previous exposure and consumption of alcohol with chronic neuropsychological symptoms.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al., 1993a	96 Danish workers involved in metal degreasing with chlorinated solvents, mostly TCE ( <i>n</i> = 70); (industries not specified), age range: 19–68; no external controls.	Chronic exposure to TCE ( <i>n</i> = 70); CFC ( <i>n</i> = 25); HC ( <i>n</i> = 1); average duration: 7.1 yrs; range of full-time degreasing: 1 mo to 36 yrs; occupational history, blood and urinary metabolites (TCA); biological monitoring for TCE and TCE metabolites; CEI calculated based on number of h/wk worked with solvents × yr of exposure × 45 wk per yr; 3 groups: (1) low exposure: <i>n</i> = 19, average full-time exposure 0.5 yr; (2) medium exposure: <i>n</i> = 36, average full-time exposure 2.1 yrs.; (3) high exposure: <i>n</i> = 41, average full-time exposure 11 yrs; Mean TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L); TWA measurements of CFC 113 levels: 260–420 ppm (U.S. and Danish TLV is 500 ppm).	Medical interview, neurological exam, neuropsychological exam; <b>Tests:</b> WAIS: Vocabulary, Digit Symbol; Simple Reaction Time, acoustic-motor function, discriminatory attention, Sentence Repetition, Paced Auditory Serial Addition Test, Text Repetition, Rey’s Auditory Verbal Learning, visual gestalt, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, Mira; Blind study.	Fisher's exact test, Chi-square trend test, t-test, ANOVA, logistic regression, odds ratios, Chi-square goodness-of-fit test; Confounders examined: age, primary intellectual level, arteriosclerosis, neurological/psychiatric disease, alcohol abuse, and present solvent exposure.	After adjusting for confounders, the high exposure group has significantly increased risk for psychoorganic syndrome following exposure (OR: 11.2); OR for medium exposed group = 5.6; Significant increase in risk with age and with decrease in WAIS Vocabulary scores; Prevalence of psychoorganic syndrome: 10.5% in low exposure group, 38.9 in medium exposure group, 63.4% in high exposure group; no significant interaction between age and solvent exposure.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al., 1993b	96 Danish workers involved in metal degreasing with chlorinated solvents (industries not specified), age range: 19–68; No external controls.	Chronic exposure to TCE ( $n = 70$ ); CFC ( $n = 25$ ); HC ( $n = 1$ ); average duration: 7.1 yrs; range of full-time degreasing: 1 mo to 36 yrs; occupational history, blood and urinary metabolites (TCA); biological monitoring for TCE and TCE metabolites; CEI calculated based on number of h/wk worked with solvents $\times$ yr of exposure $\times$ 45 wks per yr; 3 groups: (1) low exposure: $n = 19$ , average full-time expo 0.5 yr; (2) medium exposure: $n = 36$ , average full-time exposure 2.1 yrs; (3) high exposure: $n = 41$ , average full-time exposure 11 yrs; Mean TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L); TWA measurements of CFC 113 levels: 260–420 ppm (U.S. and Danish TLV is 500 ppm).	WAIS (original version): Vocabulary, Digit Symbol, Digit Span; Simple Reaction Time, Acoustic-motor function (Luria), Discriminatory attention (Luria), Sentence Repetition, PASAT, Text Repetition, Rey's Auditory Verbal Learning, Visual Gestalts, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, Mira; Blind study.	Linear regression analysis; Confounding variables analyzed: age, primary intellectual function, word blindness, education, arteriosclerosis, neurological/psychiatric disease, alcohol use, present solvent exposure.	Dose response with 9 of 15 tests; Controlling for confounds, significant relationship of exposure was found with Acoustic-motor function ( $p < 0.001$ ), PASAT ( $p < 0.001$ ), Rey AVLT ( $p < 0.001$ ), vocabulary ( $p < 0.001$ ), and visual gestalts ( $p < 0.001$ ); significant age effects.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al., 1993c	96 Danish workers involved in metal degreasing with chlorinated solvents (industries not specified), age range: 19–68; No external controls.	Chronic exposure to TCE ( $n = 70$ ); CFC ( $n = 25$ ); HC ( $n = 1$ ); average duration: 7.1 yrs; range of full-time degreasing: 1 mo to 36 yrs; occupational history, blood and urinary metabolites; biological monitoring for TCE and TCE metabolites; CEI calculated based on number of h/wk worked with solvents $\times$ yr of exposure $\times$ 45 wk per yr; 3 groups: (1) low exposure: $n = 19$ , average full-time expo 0.5 yr; (2) medium exposure: $n = 36$ , average full-time exposure 2.1 yrs; (3) high exposure: $n = 41$ , average full-time exposure 11 yrs; Mean TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L); TWA measurements of CFC 113 levels: 260–420 ppm (U.S. and Danish TLV is 500 ppm).	Medical interview, clinical neurological exam, neuropsychological exam.	Multiple regression; Fisher's exact test; Mantel-Haenzel test for linear association.	Significant dose response between exposure and motor dyscoordination remained after controlling for confounders; Bivariate analysis showed increased vibration threshold with increased exposure, but with multivariate analysis, age was a significant factor for the increase.



**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Ruijten et al., 1991	31 male printing workers exposed to TCE. Mean age 44; Mean duration 16 yrs; Controls: 28; mean age 45 yrs.	Relied on exposure data from past monitoring activities conducted by plant personnel using gas detection tubes. Estimated 17 ppm for past 3 yrs, 35 ppm for preceding 8 yrs and 70 ppm before that. Individual cumulative exposure was calculated as time spent in different exposure periods and the estimated exposure in those periods. Mean cumulative exposure = 704 ppm × yrs (SD 583, range: 160–2,150 ppm × yrs.	General questionnaire, cardiogram recorded on ink writer to measure Autonomic nerve function, including forced respiratory sinus arrhythmia (FRSA), muscle heart reflex (MHR), resting arrhythmia; Trigeminal nerve function measured using masseter reflex and blink reflex; electrophysiological testing of peripheral nerve functioning using motor nerve conduction velocity of the peroneal nerve.	Combined Z score = individual Z scores of the FRSA and MHR; ANCOVA to calculate difference between exposed/nonexposed workers; Cumulative exposure effect calculated by multiple linear regression analysis. Controlled for age, alcohol consumption, and nationality by including them as covariables. Quetelet-index included for autonomic nerve parameters; Body length and skin temperature used for all peripheral nerve functions; one-sided significance level of 5% used. Non-normal distributions were log or square root transformed.	Slight reduction in Sural nerve conduction velocity was found and a prolongation of the Sural refractory period. Latency of the masseter reflex had increased. No prolongation of the blink reflex was found; no impairment of autonomic or motor nerve function were found. Long term exposure to TCE at threshold limit values (approximately 35 ppm) may slightly affect the trigeminal and sural nerves.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Smith, 1970	130 (108 males, 22 females); Controls: 63 unexposed men working at the same factory matched by age, marital status.	TCA metabolite levels in urine were measured: 60.8% had levels up to 20 mg/L, and 82.1% had levels up to 60 mg/L.	Cornell Medical Index Questionnaire (Psychiatric section), Heron's Personality Questionnaire, Fluency Test, 13-Mistake Test, Serial Sevens, Digit Span, General Knowledge Test, tests of memory.	Descriptive Statistics.	Of the 130 subjects exposed 27% had no complaints of symptoms, 74.5% experienced fatigue, 56.2% dizziness, 17.7% headache, 25.4% gastro-intestinal problems, 7.7% autonomic effects, and 24.9% had other symptoms. The number of complaints reported by subjects were statistically significant between those with 20 mg/L or less TCA (M = 1.8 complaints) and those 60 mg/L or more (M = 2.7). Each group, however, had a similar proportion of subjects who reported having only 'slight' symptoms. The total time of continuous exposure to TCE (ranging from less than 1 yr to more than 10 yrs) appeared to have little influence on frequency of symptoms. No results of the tests are reported; Author postulates that symptom assessment raises the possibility of "errors of subjective judgment."
Triebig et al., 1977b	This study was conducted on 8 subjects occupationally exposed to TCE. Subjects were 7 men and 1 woman with an age range from 23–38 yrs. There was no control group.	Measured TCE in air averaged 50 ppm (260 mg/m <sup>3</sup> ). Length of occupational exposure was not reported.	Results were compared after exposure periods, and compared to results obtained after periods removed from exposure. TCA and TCE metabolites in urine and blood were measured. Psychological tests included d2, MWT-A, and short test.	Wilcoxon and Willcox nonparametric tests. Due to the small sample size a significance level of 1% was used.	Mean values observed were 330-mg trichloroethanol and 319-mg TCA/g creatinine, respectively, at the end of a work shift. The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig, 1982	This study was conducted on 24 healthy workers (20 males, 4 females) exposed to TCE occupationally at three different plants. The ages 17–56; length of exposure ranged from 1 to 258 mos (mean 83 mos). A control group of 144 controls used to establish ‘normal’ responses on the nerve conduction studies. The matched control group consisted of 24 healthy nonexposed individuals (20 males, 4 females), chosen to match the subjects for age and sex.	Length of exposure ranged from 1 to 258 mos (mean 83 mos). TCE concentrations measured in air at work places ranged from 5–70 ppm. TCA, TCE, and trichloroethanol were measured in blood, and TCE and TCA were measured in urine.	Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: MCV <sub>MAX</sub> (U): Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; dSCV (U), pSCV (U), and dSCV (M).	Data were analyzed using parametric and nonparametric tests, rank correlation, linear regression, with 5% error probability.	Results show no statistically significant difference in nerve conduction velocities between the exposed and unexposed groups. This study has measured exposure data, but exposures/responses are not reported by dose levels.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig, 1983	The exposed group consists of 66 healthy workers selected from a population of 112 workers. Workers were excluded based on polyneuropathy ( $n = 46$ ) and alcohol consumption ( $n = 28$ ). The control group consisted of 66 healthy workers with no exposures to solvents.	Subjects were exposed to a mixture of solvents, including TCE, specifically “ethanol, ethyl acetate, aliphatic hydrocarbons (gasoline), MEK, toluene, and trichloroethene.” Subjects were divided into 3 exposure groups based on length of exposure, as follows: 20 employees with “short-term exposure” (7–24 mos); 24 employees with “medium-term exposure” (25–60 mos); 22 employees with “long-term exposure” (over 60 mos). TCA, TCE, and trichloroethanol were measured in blood, and TCE and TCA were measured in urine.	Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: $MCV_{MAX}(U)$ : Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; $dSCV(U)$ , $pSCV(U)$ , and $dSCV(M)$ .	Data were analyzed using parametric and nonparametric tests, rank correlation, linear regression, with 5% error probability.	There was a dose-response relationship observed between length of exposure to mixed solvents and statistically significant reduction in nerve conduction velocities observed for the medium and long-term exposure groups for the NCV.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

<b>Reference</b>	<b>Study population</b>	<b>Exposure assessment and biomarkers</b>	<b>Tests used</b>	<b>Statistics</b>	<b>Results</b>
Troster and Ruff, 1990	3 occupationally exposed workers to TCE or TCA; 2 patients acutely exposed to low levels of TCE and 1 patient exposed to TCA; Controls: 2 groups of $n = 30$ matched controls; (all age and education matched).	“Unknown amount of TCE for 8 months.”	SDNTB, “1 or more of:” TAT, MMPI, Rorschach, and Interviewing questionnaire, Medical examinations (including neurological, CT scan, and/or Chemo-pathological tests and occupational history).	Not reported.	Case 1: Intelligence “deemed” to drop from premorbid function at 1 y 10 mos after exposure. Impaired functions improved for all but reading comprehension, visuospatial learning and categorization (abstraction). Case 2: Mild deficits in motor speed, verbal learning, and memory; “marked” deficits in visuospatial learning; good attention; diagnosis of mild depression and adjustment disorder, but symptoms subsided after removal from exposure. Case 3: Manual dexterity and logical thinking borderline impaired; no emotional changes, cognitive function spared, diagnosis of somatoform disorder.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
White et al., 1997	<p>Group 1: 28 individuals in Massachusetts exposed to contaminated well water; source: tanning factory and chemical plant; age range: 9–55.</p> <p>Group 2: 12 individuals in Ohio exposed to contaminated well water; source: degreasing; age range: 12–68</p> <p>Group 3: 20 individuals in Minnesota exposed to contaminated well water; <i>n</i> = 14 for nerve conduction studies and <i>n</i> = 6 for neuropsychological testing; source: ammunition plant; age range: 8–62. No controls.</p>	<p>Group 1: 2 wells tested in 1979: 267 ppb TCE, 21 ppb Tetrachloroethylene, 12 ppb chloroform, 29 ppb dichloroethylene, 23 ppb Trichlorotrifluoroethane; 2 yrs average TCE 256 ppb for well G, and 111 ppb for well H.</p> <p>Group 2: 13 wells with 1,1,1-trichloroethane (up to 2,569 ppb) and TCE (up to 760 ppb); blood analysis of individuals 2 yrs after end of exposure and soon after exposure showed normal or mild elevations of TCE, elevations of 1,1,1-trichloroethane, ethylbenzene, and xylenes. Group 3: mean TCE for one well 261 ppb; 1,1-dichloroethylene 9.0 ppb; 1,2-dichloroethylene 107 ppb.</p>	<p>Occupational and environmental questionnaire, neurological exam, neuropsychological exam: WAIS-R, WISC-R, WMS, WMS-R, Wisconsin Card Sorting, COWAT, Boston Naming, Boston Visuospatial Quantitative Battery, Milner Facial Recognition Test, Sticks Visuospatial Orientation Task, Word triads, Benton Visual Retention Test, Santa Ana, Albert's Famous Faces, Peabody Picture Vocabulary Test, WRAT, POMS, MMPI, Trail-making, Fingertapping, Delayed Recognition Span Test; Neurophysiological exam: eyeblink, evoked potentials, nerve conduction; Other: EKG, EEG, medical tests.</p>	<p>Data shown in proportion in 3 communities, clinical diagnostic categories, analysis of central tendencies, and descriptive statistics.</p>	<p>Group 1: Some individuals with subclinical peripheral neuropathy; 92.8% with reflex abnormalities; 75% total diagnosed with peripheral neuropathy; 88.9% with impairment in at least 1 memory test; Impairments: attention and executive function in 67.9%; motor function in 60.71%, visuospatial in 60.71%, mild to moderate encephalopathy in 85.7%.</p> <p>Group 2: 25% with abnormal nerve conduction; Impairments: attention and executive function in 83.33%, memory in 58.33%, language/verbal in 50%.</p> <p>Group 3: 35.7% with peripheral neuropathy; neuropsychological: all 6 tested had memory impairment, attention and executive function impairment, 3 had manual motor slowing. Participants younger at time of exposure with wider range of deficits; Language deficits in younger, but not in older participants.</p>

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Winneke, 1982	<p>This is a review article presenting multiple studies that evaluated neurological effects of TCE, and other solvents. Only the TCE results are summarized herein.</p> <p>Experiment 1: 18 subjects (results taken from Schlipkoter et al., 1974 and summary is based on informations from Winneke, 1982)</p> <p>Experiment 2: 12 subjects (results taken from Winneke et al., 1974, 1976, 1978 and summary is based on information from Winneke, 1982)</p>	<p>Experiment 1: Subjects were exposed to 50 ppm TCE for 3.5 hours</p> <p>Experiment 2: Comparative study of effects from (a) 50 ppm TCE for 3.5 hours and (b) 0.76 ml/kg ethanol.</p>	For both experiments 1 and 2: critical flicker fusion, sustained attention task, auditory evoked potentials	No statistical details were reported.	Significant decrease ( $p < 0.05$ ) in auditory evoked potentials in individuals (experiments 1 and 2) exposed to 50 ppm TCE. No significant effects were noted in the critical flicker fusion or the sustained attention tasks.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
ATSDR, 2003	116 children from registry of 14 hazardous waste sites with TCE in groundwater; under 10 yrs of age at time of registry; Control population ( <i>n</i> = 177); communities with no evidence of TCE in groundwater (measured below MCL); matched by age and race; there were other chlorinated solvents present in the exposed group wells.	Exposures were modeled using tap water TCE concentrations and GIS for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences, modeled data were used to estimate lifetime exposures (ppb-yrs) to TCE in residential wells; 3 exposure level groups; control = 0 ppb; low exposure group = 0 <23 ppb-yrs; and high exposure group = >23 ppb-yrs; confounding exposure was a concern.	Fisher Logemann test; OSME-R; CSP; D-COME-T; hearing screening; DPOAE; SCAN.	Screening results as binary variables using logistic regression within SAS; independent variables included exposure measures, age, gender, case history; chi-square test, Fisher's exact test, t-tests, linear models.	Exposed children had higher abnormalities for D-COME-T ( <i>p</i> < 0.002), CSP ( <i>p</i> < 0.008), velopharyngeal function ( <i>p</i> < 0.04), high palatal arch ( <i>p</i> < 0.04), abnormal outer ear cochlear function; No difference observed in exposed and nonexposed populations for speech or hearing function; No difference found in OSH function.



**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
<b>Epidemiological Studies: Controlled Exposure Studies; Neurological Effects of Trichloroethylene</b>					
Gamberale et al., 1976	15 healthy men aged 20–31-yr old employed by the Department of Occupational Medicine in Stockholm, Sweden; Controls: Within Subjects (15 self-controls).	Exposed for TCE 70 mins via a breathing valve to 540 mg/m <sup>3</sup> (97 ppm), 1,080 mg/m <sup>3</sup> (194 ppm), and during ordinary atmospheric air. Sequence was counterbalanced between the 3 groups, days, and exposure levels. Concentration was measured with a gas chromatographic technique every third min for the 1 <sup>st</sup> 50 mins, then between tests thereafter.	RT addition, simple RT, choice RT and short term memory using an electronic panel. Subjects also assessed their own conditions on a 7-pt scale.	Friedman two-way analysis by ranks to evaluate difference between 3 conditions, nonsignificant when tested individually, but significant when tested on the basis of 6 variables. Nearly half of the subjects could distinguish exposure/nonexposure. ANOVA for 4 performance tests based on a 3 × 3 Latin square design with repeated measures.	In the RT-Addition test the level of performance varied significantly between the different exposure conditions (F[2.24] = 4.35; <i>p</i> < 0.051) and between successive measurement occasions (tF[2.24] = 19.25; <i>p</i> < 0.001); The level of performance declined with increased exposure to TCE, whereas repetition of the testing led to a pronounced improvement in performance as a result of the training effect; No significant interaction effects between exposure to TCE and training.
Konietzko et al., 1975	This is a controlled exposure study conducted on 20 healthy male students and scientific assistants with a mean age of 27.2 yrs.	Subjects were exposed to a constant TCE concentration of 95.3 ppm (520 mg/m <sup>3</sup> ) for up to 12 h, and Blood concentrations of TCE were also analyzed at hourly intervals.	Evaluated for changes in alpha waves (<14 Hz) in the EEG recordings; EEG recordings were performed hourly for a period of 1 min with the eyes closed. This was used as a potential measure of psychomotor disturbance.		The alpha segment increased over time of exposure (from 0800 to 0900 and 1000 h [military time]) (P = 0.05). There were no significant differences for the other time spans or for other parameters. Subjects with highest and lowest TCE blood levels <2 µg/mL and >5 µg/mL were compared to determine if they showed different responses, but no case were the differences statistically different.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kylin et al., 1967	12 subjects exposed to 1,000 ppm TCE for 2 h in a 1.5 × 2 × 2 meters chamber; 2 subjects were given alcohol (0.7 gm of body weight); Controls: 7 of the 12 were tested some days prior to exposure and 5 of the 12 were tested some days after exposure.	1,000 ppm of TCE was blown into a chamber via an infusion unit and vaporizing system. Ostwald's distribution factor for TCE—the quotient of the amount of solvent in the blood by the amount of alveolar air.	Optokinetic Nystagmus; Venus blood and alveolar air specimens were taken at various times after exposure and analyzed in a gas chromatograph with a flame ionization detector.	Ostwald's distribution factor for TCE (the quotient of the amount of solvent in the blood in mg/L by the amount of the alveolar air in mg/L) = 9.7; Significant relationship between TCE in air and blood (0.88).	"A number" of subjects showed reduction in Fusion limit although more pronounced in the 2 subjects who consumed alcohol. "Others," however, showed little if any effect. No stats.
Salvini, 1971	This is a controlled exposure study conducted on 6 male university students. Each subject was examined on 2 different days, once under TCE exposure, and once as self controls, with no exposure.	TCE concentration was 110 ppm for 4-h intervals, twice per day. 0-ppm control exposure for all as self controls.	Two sets of tests were performed for each subject corresponding to exposure and control conditions. Perception test with tachistoscopic presentation, Wechsler memory scale, complex reaction time test (CRT), and manual dexterity test.	ANOVA	A decrease in function for all measured effects was observed. Statistically significant results were observed for perception tests learning ( $p < 0.001$ ), mental fatigue ( $p < 0.01$ ), subjects ( $p < 0.05$ ); and CRT learning ( $p < 0.01$ ), mental fatigue ( $p < 0.01$ ), subjects ( $p < 0.05$ ).

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Stewart et al., 1970	13 subjects in 10 experiments	Ten chamber exposures to TCE vapor (100 ppm and 200 ppm) for periods of 1 h to a 5-day work week. Experiments 1–7 were for a duration of 7 h with a mean TCE concentration of 198–200 ppm. Experiments 8 and 9 exposed subjects to 202 ppm TCE for a duration of 3.5 and 1 h, respectively. Experiment 10 exposed subjects to 100 ppm TCE for 4 h. Experiments 2–6 were carried out with the same subjects over 5 consecutive days; Gas chromatography of expired air; No self controls.	Physical examination 1 h prior to exposure. Blood analysis for complete blood cell count (CBC), sedimentation rate, total serum lipid, total serum protein, serum electrophoresis, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase. 24-h urine collection for urobilinogen, TCA and TCE. Also a preexposure expirogram, tidal volume measurement, and an alveolar breath sample for TCE; Short neurological exam including modified Romberg test, heel-to-toe test, finger-to-nose test.	Descriptive statistics.	Ability to perceive TCE odor diminished as duration of expo increased; 40% had dry throat after 30 min. exposure; 20% reported eye irritation; Urine specimens showed progressive increase in amounts of TCE metabolites over the 5 consecutive exposures. Concentrations of TCA and TCE decreased exponentially after last exposure, but still present in abnormal amounts in urine specimens 12 d after exposure. Loss of smelling TCE: >1 h = 33%; >2 h = 80%; >6.5 h = 100%; Symptoms of lightheadedness, headache, eye, nose and throat irritation. Prominent fatigue and sleepiness by all after 200 ppm. These symptoms may be of clinical significance. All had normal neurological tests during exposure, but 50% reported greater mental effort was required to perform a normal modified Romberg test on more than one occasion.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig, 1976	This was a controlled exposure study conducted on 7 healthy male and female students (4 females, 3 males). The control group was 7 healthy students (4 females, 3 males).	Subjects exposed for 6 h/d for 5 d to 100 ppm (550 mg/m <sup>3</sup> TCE). Controls were exposed in chamber to zero TCE. Biochemical tests included TCE, TCE, and trichloroethanol in blood. In this study the TCE concentrations in blood reported ranged from 4 to 14 µg/mL. A range of 20 to 60 µg/mL was obtained for TCA in the blood.	Psychological tests were: the d2 test was an attention load test; the short test is used to record patient performance with respect to memory and attention; <u>daily Fluctuation Questionnaire</u> measured the difference between mental states at the start of exposure and after the end of exposure is recorded; The MWT-A is a repeatable short intelligence test; the Freiburg Personality Inventory is a test for 12 independent personality traits; CFT-3 is a nonverbal intelligence test; Erlanger Depression Scale.	Regression analyses were conducted.	There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. The biochemical data did demonstrate that exposed subjects' exposures.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig et al., 1977a	This was a controlled exposure study conducted on 7 healthy male and female students (4 females, 3 males) The control group was 7 healthy students (4 females, 3 males).	Subjects exposed for 6 h/d for 5 days to 100 ppm (550 mg/m <sup>3</sup> TCE). Controls were exposed in chamber to zero TCE. Biochemical tests included TCE, TCA and trichloroethanol in blood. In this study the TCE concentrations in blood reported ranged from 4 to 14 µg/mL. A range of 20 to 60 µg/mL was obtained for TCA in the blood.	The testing consisted of: the Syndrome Short Test; the “Attention Load Test” or “d2 Test;” Number recall test, letter recall test, The “Letter Reading Test;” “Word Reading Test;” Erlanger Depression Scale. Scale for Autonomic Dysfunction, Anxiety Scale, Pain Short Scale, and Information on Daily Fluctuations.	Statistics were conducted using Whitney Mann.	Results indicated the anxiety values of the placebo random sample group dropped significantly more during the course of testing ( $p < 0.05$ ) than those of the active random sample group. No significantly different changes were obtained with any of the other variables.
Vernon and Ferguson, 1969	8 male volunteers age range 21–30; self controls: 0 dose.	TCE administered as Trilene air-vapor mixtures through spirometers administered at random concentrations of 0, 100, 300, or 1,000 ppm of TCE for 2 h at a time, during which testing took place. Concentrations were measured with a halide meter. Medical history, exam including CBC, urinalysis, BUN, and SGOT.	Flicker Fusion with Krasno-Ivy Flicker Photometer, Howard-Dolman depth perception apparatus, Muller-Lyer two-dimensional illusion, groove-type steadiness test, Purdue Pegboard, Written “code substitution,” blood studies.	ANOVAs, Dunnett's test.	TCE did not produce any appreciable effects at lower concentrations. Compared to controls, participants exposed to 1,000 ppm of TCE had adverse effects on the Howard-Dolman, steadiness, and part of the pegboard, but no effects on Flicker Fusion, from perception or code substitution. No appreciable changes in CBC, urinalysis, SGOT, or BUN.

**Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Windemuller and Ettema, 1978	Pilot study: 24 healthy male volunteers; age range = 19–26 yr, 4 groups with 6 volunteers in each: (1) control, (2) exposed to TCE, (3) exposed to alcohol, (4) exposed to TCE and alcohol; Final study: 15 other volunteers, each exposed to all 4 conditions.	Chamber study; Group 1 no exposure; Group 2 TCE exposure: 2.5 h with 200 ppm; Group 3 alcohol exposure: 0.35 g/kg body weight; Group 4 TCE and alcohol: same as above levels; Blood alcohol levels taken with breathalyzer; exhaled air sampled for levels of TCE and trichloroethanol; TCE exposure: average measured TCE in exhaled air = 29 µg/L (SD = 3); TCE and alcohol expo: average measured TCE in exhaled air = 63 µg/L (SD = 12).	Binary Choice Task (Visual); Pursuit Rotor; Recording of heart rate, sinus arrhythmia, breathing rate; Questionnaire (15 items on subjective feelings).	K-sample trend test; two-tailed Wilcoxon test.	Pilot study: no systematic effect of exposure on test perform. Alcohol group had higher heart rate than TCE group, and TCE and alcohol group; minimal effect of mental load on heart rate; sinus arrhythmia suppressed as mental load increased with higher suppression in exposed groups (all 3) compared to controls (differences possibly due to existing group differences); Final Study: pursuit-rotor task “somewhat impaired by exposure condition;” authors acknowledge possibility of sequence effects; no significant difference between conditions on questionnaire responses; performing mental tasks resulted in higher heart rate in the TCE + alcohol condition than in Alcohol alone condition; Mental load suppressed sinus arrhythmia, especially in TCE + alcohol condition; Conclusion: TCE and alcohol together impair mental capacity more than each one alone.

BUN = blood urea nitrogen, EEG = electroencephalograph, GI = gastrointestinal, NIOSH = National Institute of Occupational Safety and Health, OR = odds ratio, PCE = perchloroethylene.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Albers et al., 1999	30 railroad workers with toxic encephalopathy; involved in litigation; long-term exposure to solvents ( $n = 20$ yrs.; range = 10–29 yrs.); Historical controls matched by gender, age, and body mass.	Most common solvents included trichloroethylene, trichloroethane, perchloroethylene; respirator not typically used.	Neurologic exams (cranial nerves, motor function, alternate motion range, subjective sensory function, Romberg test, reflexes), occupational history, medical history, sensory and motor nerve conduction studies (NCS).	Log transformations of amplitude data; Mann-Whitney U Test for NCS; t-test; simple linear regression and stepwise regression for dose response.	3 workers met clinical polyneuropathy criteria; NCS values not influenced by exposure duration or job title; no significant difference in NCS between presence or absence of polyneuropathy symptoms, disability status, severity or type of encephalopathy, or prior polyneuropathy diagnosis.
Antti-Poika, 1982	87 patients (painters, paint and furniture factory workers, carpet and laundry workers) diagnosed 3–9 yrs prior with chronic solvent exposure (mean age 38.6 yrs) Control: 29 patients with occupational asthma.	Mean duration of exposure 10.4 yrs; solvents: trichloroethylene, perchloroethylene, solvent mixture; based on patients' and/or employers' reports; 9 worksites visited for environmental measures; biological measures at 1 worksite; exposure classified as low, moderate, or high.	Interview, Neurologic exam, EEG, electroneuromyographs, psychological examination (intellectual, short-term memory, sensory and motor functions).	Correlation coefficients for prognosis and factors influencing diagnosis.	Reported symptoms: fatigue, headaches, memory disturbances, pain, numbness, paresthesias; 1 <sup>st</sup> exam: 87 patients with objective and subjective neurological signs, 61 with psychological disturbance, 58 abnormal EEG, 25 clinical abnormalities, 57 PNS symptoms; 69 patients had neurophysiological or psychological disturbances identified by neurologist in only 4 patients; 2 <sup>nd</sup> exam: 42 with clinical neurological signs, ; 21 patients deteriorated, 23 improved, 43 same; poor correlation between prognosis of examinations; no significant correlation between prognosis and age, sex, exposure duration and level, alcohol use, or other diseases.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Aratani et al., 1993	437 exposed workers from various industries (not specified); 394 males, 43 females and 1,030 male clerical workers as controls; age range: 16–72.	Exposed to Thinner, G/5100, TCE, xylene, toluene, methylchloride, gasoline.	Vibrometer (VPT); Urinary Metabolites.	Spearman correlation.	Positive correlations between age and VPT 7; between job experience and VPT; Urinary metabolites not significantly correlated with VPT; no dose-effect for subjective symptoms and neurological signs.
Binaschi and Cantu, 1982	35 patients with occupational exposure to organic solvents; Industry not specified; no controls.	Occupational history provided by patients; Descriptions of jobs and conditions provided by employer; Workplace observations; Some available measurements of solvents in air; 9 patients exposed to trichloroethylene; 11 exposed to toluene and xylene; 15 exposed to mixtures of solvents; all exposures described to be under TLV-TWA, but short exposure might have exceeded ACGIF limit for short time.	Examination of provoked and spontaneous vestibular symptoms; Pure tone threshold measurement; EEG; psychiatric interviews and psychiatric history; Prevalence of 37 psychiatric symptoms.	Not stated.	All patients had subjective symptoms (fatigue, psychic disturbances, dizziness, vegetative symptoms, vertigo); Vestibular system affected in most cases, with lesions in nucleo-reticular substance and brain stem; EEG change with diffuse and focal slowing; 71% of patients had mild neurasthenic symptoms (fatigue, emotional instability, memory and concentration difficulties).



**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Bowler et al., 1991	67 former microelectronics workers exposed to multiple organic solvents; Controls ( $n = 157$ ) were recruited from the same region; 67 pairs were matched on the basis of age, sex, ethnicity, educational level, sex, and number of children.	Self-report and work history from microelectronics workers. Exposures and risks were estimated. Solvents include TCE, TCA, benzene, toluene, methylene chloride, n-hexane.	California Neuropsychological Screening Battery.	t-test for matched pairs; Wilcoxon Signed Rank test.	Exposed workers performed significantly worse on tests of attention, verbal ability, memory, visuospatial, visuomotor speed, cognitive flexibility, psychomotor speed, and reaction time; no significant differences in mental status, visual recall, learning, and tactile function.
Colvin et al., 1993	Final sample: 67 workers (43 exposed; 24 unexposed) in a paint manufacturing plant employed there for at least 5 yrs.; all black males; exclusion criteria: encephalopathy, head injury with 24 + h unconsciousness, psychotropic medication, alcohol/drug dependence history, epilepsy, mental illness.	Chronic exposure was assessed through self-reported detailed work history for each worker; past and current industrial hygiene measurements of solvent levels in air; “total cumulative expo” in the factory and “average lifetime exposures” were calculated; visitations to establish areas with “homogeneous exposure;” All exposures below the ACGIH limit. Solvents include MEK, benzene, TCE, MIBK, toluene, butyl acetate, xylene, cellosolve acetate, isophorone, and white spirits.	Work and personal history interview; brief neurological evaluation, WHO Neurobehavioral Core Test Battery (all tests except POMS); Computer-administered tests: Reaction time, Fingertapping, Continuous Performance Test, Switching attention, Pattern Recognition Test, Pattern Memory; UNISA Neuropsychological Assessment Procedure: Four word memory test, Paragraph memory, Geometric Shape drawing; symptom and health questionnaires.	Division into exposed and unexposed; Student’s t-test; Multiple linear regression.	Exposed group performed worse than unexposed on 27 out of 33 test results; only significant difference was on latency times of two switching attention tests; no difference in subjects’ symptom reporting between groups when questions analyzed separately or analyzed as a group; Average lifetime exposure was a significant predictor for Continuous performance latency time, Switching attention latency time, Mean reaction time, Pattern Memory; fine visuomotor tracking speed significantly associated with cumulative exposure; effects of exposure concluded to be “relatively mild” and subclinical.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

<b>Reference</b>	<b>Study population</b>	<b>Exposure assessment and biomarkers</b>	<b>Tests used</b>	<b>Statistics</b>	<b>Results</b>
Daniell et al., 1999	89 retired male workers (62–74-yr old) with prior long-term exposure to solvents including 67 retired painters and 22 aerospace manufacturing workers; Controls: 126 retired carpenters with minimal solvent exposure.	Chronic occupational exposure; Structured clinical interview about past and present exposure to solvents; Cumulative Exposure Index was constructed. Solvents not specified.	Psychiatric interview; questionnaires; physical exam; blood cell counts, chemistry panel, blood lead levels, Neuropsychological: BDI, verbal fluency test, WAIS-R: Vocabulary, Similarities, Block Design, Digit Span, Digit Symbol; Wisconsin Card Sorting; verbal aphasia screening test, Trails A and B, Fingertapping; WMS-R: logical memory and visual subtests; Rey Auditory Verbal Learning; Benton Visual Retention test; d2 test; Stroop; Grooved pegboard; simple reaction time.	Odds ratio, logarithmic transformation of non-Gaussian data, standardization of test scores, ANCOVA, Multiple Linear regression; Kruskal Wallis test for differences in blood lead concentration.	CEI was similar for painters and aerospace workers; Painters reported greater alcohol use than carpenters; painters also had lower scores on WAIS-R Vocabulary subtest; Controlling for age, education, alcohol use, and vocabulary score, painters performed worse on motor, memory, and reasoning ability tests; painters reported more symptoms of depression and neurological symptoms; painters more likely to have more abnormal test scores (odds ratio: 3.1) as did aerospace workers (odds ratio: 5.6); no dose effect with increasing exposure and neuropsychological tests.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Donoghue et al., 1995	16 patients diagnosed with organic-solvent-induced toxic encephalopathy with various occupations compared to age-stratified normal groups ( $n = 38$ ); average age: 43 y (range = 31–58); Exclusion criteria: diabetes mellitus, ocular disease impairing vision, visual acuity with existing refractive correction of less than 4/6, abnormal direct ophthalmoscopic exam.	Average exposure duration was 19 yrs (range = 5–36 yrs); Solvents include TCE, MEK, toluene, thinners, unidentified hydrocarbons.	Visual acuity measured with a 4-m optotype chart; Contrast sensitivity measured with Vistech VCTS 6500 chart; monocular thresholds, pupil diameter.	Chi-square test.	6 participants (37.5%) with abnormal contrast sensitivity; 2 of the 6 (33%) had monocular abnormalities; abnormalities occurred at all tested spatial frequencies; significant difference between groups at 3 cpd, 6 cpd, 12 cpd frequencies.
Elofsson et al., 1980	Epidemiologic study of car or industrial spray painters (male) exposed long-term to low levels of organic solvents ( $n = 80$ ); 2 groups of matched controls; 80 nonexposed male industrial workers in each control group.	Long term, low level expo to multiple solvents; Assessed by interviews, on-the-job measurements, and a 1955 workshop model; Blood analysis: mean values were within normal limits for both groups; Exposed group had significantly higher values for alkaline phosphates, hemoglobin, hematocrit, and erythrocytes; early exposure TLVs in Sweden were significantly lower; solvents include TCE, TCA, methylene chloride, and others.	Self-administered psychiatric questionnaires, Eysenck's Personality Inventory, psychosocial structured interview, Comprehensive Psychopathological Rating Scale; Visual Evoked Responses; EEG; Electroneurography; Vibration Sense Threshold estimations; Neurological exam.	Calculation of z values; Pearson correlation; Multiple Regression Analysis.	Significant differences between controls and exposed in symptoms of neurasthenic syndrome, in reaction time, manual dexterity, perceptual speed, and short-term memory; no significant differences on verbal, spatial, and reasoning ability; Some differences on EEG, VER, ophthalmologic, and CT.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Gregersen, 1988	Workers exposed to organic solvents (paint, lacquer, photogravure, and polyester boat industries); Controls: warehousemen electricians; 1 <sup>st</sup> follow-up 5.5 yrs after initial evaluation (59 exposed, 30 unexposed); 2 <sup>nd</sup> follow-up: 10.6 yrs after initial evaluation (53 exposed, 30 unexposed controls).	1 <sup>st</sup> follow-up: data about working conditions, materials and exposure in prior 5 yrs used for exposure index; 2 <sup>nd</sup> follow-up: 9 questions asking about exposure to solvents in the prior 5 yrs; TCE, toluene, styrene, white spirits.	1 <sup>st</sup> follow-up: structured interviews on occupational, social, medical history; clinical exam, neurological exam; 2 <sup>nd</sup> follow-up: mailed questionnaire (49 follow-up issues to 1 <sup>st</sup> follow-up).	Wilcoxon-Mann-Whitney tests; Kruskal-Wallis test; Chi-square; Spearman Rank Partial Correlation Coefficient.	More acute neurotoxic symptoms in exposed group at both follow-ups, but fewer symptoms at 2 <sup>nd</sup> follow-up than at 1 <sup>st</sup> follow-up; at both follow-ups exposed participants had more encephalopathy symptoms, especially memory and concentration; no encephalopathy symptoms in control group; symptoms and signs of peripheral, sensory, and motor neuropathy significantly worse in participants still exposed; Exposure index showed dose-effect with memory and concentration; Both follow-ups: improvement in acute symptoms; aggravation in CNS; more symptoms of peripheral nervous system and social consequences.
Juntunen et al., 1980	37 patients with suspected organic solvent poisoning (mean age = 40.1 yrs.); selection based on pneumoencephalography; no controls.	Patients were exposed to Carbon disulphide ( $n = 6$ ), trichloroethylene (5), styrene (1), thinner (2), toluene (1), methanol (1), and carbon tetrachloride (2), mixtures (19); Exposure was assessed by patients' and employers' reports and measurements of air concentrations when available.	Neurologic examination, pneumoencephalographic exam, EEG, tests assessing intelligence, memory and learning, motor function, and personality.	Descriptive Statistics.	Clinical neurological findings of slight psychoorganic alterations, cerebellar dysfunction, and peripheral neuropathy; 63% had indication of brain atrophy; 23 of the 28 patients examined with electroneuromyography showed signs of peripheral neuropathy; 94% had personality changes, 80% had psychomotor deficits, 69% had impaired memory, and 57% had intelligence findings; No dose-effect found.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Juntunen et al., 1982	80 (41 women, 39 men) Finnish patients diagnosed 3–9 yrs prior with chronic solvent exposure (mean age = 38.6 yrs); 31 had slight neurological signs; no controls.	Assessed by patients' occupational history, employers' workplace description, observations and data collected at workplace, environmental measurements, biological tests; TCE, PCE, or mixed solvent exposures.	Neurologic examination; EEG and ENMG; tests of intellectual function, memory, learning, personality and psychomotor performance.	Chi-square, Maxwell-Stuart, Correlation and multiple linear regression analyses.	Significant correlations between prognosis of disturbances in gait ( $p < 0.05$ ) and station and length of follow-up, duration and level of exposure and multiplying the two; no gender effects; Common subjective symptoms; headaches, fatigue, and memory problems; Impairment in fine motor skills, gait, and cerebellar functions; Subjective symptoms decreased during follow-up, but clinical signs increased.
Laslo-Baker et al., 2004	32 mothers with occupational exposure to organic solvents during pregnancy and their children (3–9 yrs of age); included if exposure started in 1 <sup>st</sup> trimester and lasted for at least 8 wks of pregnancy (32 mother-child pairs); Controls: 32 unexposed control mothers matched on age, child age, child sex, SES, and reported cigarette use and their children (32 mother-child pairs).	Exposure information collected at 3 times: (1) during pregnancy, (2) when contacted for study participation later in pregnancy, (3) at time of assessment; Information collected included types of solvent, types of setting, duration of exposure during pregnancy, use of protection, symptoms, ventilation; Solvents include toluene ( $n = 12$ women), xylene (10), ethanol (7), acetone (6), methanol (5), TCE (3), etc. (a total of 78 solvents were reported).	Children: Wechsler Preschool and Primary Scale of Intelligence, WISC, Preschool Language Scale, Clinical Evaluations of Language Fundamentals, Beery-Buktenica Developmental test of Visuo-Motor Integration, Grooved Pegboard Test, Child Behavior Checklist (Parent Version), Connor's Rating Scale-Revised (Parent Version), Behavioral Style Questionnaire; Mothers: WASI.	Power analysis, Multiple linear regression.	Verbal IQ was lower (104) in children exposed <i>in utero</i> vs. unexposed children controls (110); Children did not differ between groups in birth weight, gestational age, or developmental milestones; Children in the exposed group had significantly lower VIQ (108) and Full IQ (108) than controls (VIQ = 116 and Full IQ = 114; No significant difference in PIQ; Performance on expressive language, total language, and receptive language was significantly worse in children from exposed group.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Lee et al., 1998	40 Korean female shoe factory workers employed there for at least 5 yrs.; cases with head injury, neurological or psychological disorder, or hearing or visual impairment were excluded; Controls: 28 (housekeepers); no in-plant controls available.	4 workers wore passive personal air samplers for a full 8-h shift; Detected solvents: toluene, methyl ethyl ketone, <i>n</i> -hexane, <i>c</i> -hexane, cyclohexane, dichloroethylene, trichloroethylene, benzene, and xylene; In frame-making air concentration of solvents was 0.46–0.71; In adhesive process solvent air concentrations were 1.83–2.39; three exposure indices were calculated: current exposures, exposure duration (yrs), and Cumulative Exposure Estimate (CEE) (yrs × average exposures).	Questionnaire; Neurobehavioral Core Test Battery (includes POMS, Simple Reaction Time, Santa Ana Dexterity test, Digit Span, Benton Visual Retention Test, Pursuit aiming motor steadiness test); POMS was excluded because of cultural inapplicability.	Multivariate ANOVA for tests with 2 outcomes; ANOVA for tests with 1 outcome; education was adjusted in analyses.	Significant differences between groups based on exposure index; Differences in performance between controls and participants on Santa Ana were found only in the CEE (participants performed worse); CEE is a more sensitive measure of exposure to organic solvents.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Lindstrom, 1973	168 male workers with suspected occupational exposure to solvents Group I with solvent poisoning ( $n = 42$ ); Group II with solvent exposure, undergoing mandatory periodic health check ( $n = 126$ ); Control-50 healthy nonexposed male volunteers working in a viscose factory; Group IV 50 male workers with carbon disulfide poisoning.	44 exposed to TCE, 8 to tetrachloroethylene, 26 to toluene, 25 to toluene and xylene, 44 to thinners, 21 to “miscellaneous;” Solvent-exposed group had an average of 6 y of expo; CS <sub>2</sub> group had average of 9 yrs of exposure.	WAIS: Similarities, Picture Completion, Digit Symbol; Bourdon-Wiersma vigilance test, Santa Ana, Rorschach Inkblot test, Mira test.	Student’s t-test.	The solvent-exposed group and CS <sub>2</sub> group had significantly worse “psychological performances” than controls; Greatest differences in sensorimotor speed and psychomotor function; solvent-exposed and CS <sub>2</sub> groups had deteriorated visual accuracy.
Lindstrom, 1980	56 male workers diagnosed with occupational disease caused by solvents; Controls: 98 styrene-exposed workers; 43 nonexposed construction workers.	Chronic “excessive” exposure: Mean duration of exposure = 9.1 yrs (SD = 8.3); Exposed to; halogenated and aromatic hydrocarbons, paint solvents, alcohols, and aliphatic hydrocarbons (TCE $n = 14$ ); Individual exposure levels estimated as time-weighted averages, based on information provided by subjects, employer, or workplace measurements, were categorized as low (3 patients), intermediate (26 patients), and high (27 patients).	WAIS subtests: Similarities, Digit Span, Digit Symbol, Picture Completion, Block Design; WMS subtests: Visual Reproduction; Benton Visual Retention test; Symmetry Drawing; Santa Ana Dexterity test; Mira test.	Factor analysis; Student’s t-test; Multivariate Discriminant analysis.	Significant decline in visuomotor performance and freedom from distractibility (attention) in the solvent-exposed participants; significant relationship between duration of solvent exposure and visuomotor performance; solvent exposure level was not significant; psychological test performance of styrene-exposed control was only slightly different from nonexposed controls.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

<b>Reference</b>	<b>Study population</b>	<b>Exposure assessment and biomarkers</b>	<b>Tests used</b>	<b>Statistics</b>	<b>Results</b>
Lindstrom et al., 1982	86 Patients with prior diagnosis of solvent intoxication (mean age 38.6 yrs.); 40 male, 46 female; 52 exposed to mixed solvents; 21 exposed to TCE or PCE; 13 exposed to both; results at follow-up compared to those at initial diagnosis.	Mean duration of exposure 10.4 yrs; solvents: trichloroethylene, perchloroethylene, solvent mixture; based on patients' and/or employers' reports.	Intellectual Function: from WAIS – Similarities, Block Design, Picture Completion; Short Term Memory: from WMS – Digit Span, Logical Memory, Visual Reproduction; Benton Visual Retention test; Sensory and Motor Functions: Bourdon Wiersma Vigilance Test, Symmetry Drawing, Santa Ana Dexterity test, Mira test.	Frequency distributions, Student's t-test for paired data, stepwise linear regression.	All patients grouped together regardless of types of past solvent exposure; on follow-up, significant learning effects for Similarities when compared to results at initial diagnosis; group mean for intellectual functioning increased; no significant change in memory test results; group means for sensory and motor tasks were lower; prognosis was better for longer follow-up and younger age and poorer for users of medicines with neurological effects.



**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Marshall et al., 1997	All singleton births in 1983–1986 in 188 New York State counties (total number not specified); 473 CNS-defect births and 3,305 musculoskeletal-defect births; Controls: 12,436 normal births; Exclusion criteria: Trisomy 13, 18, or 21, birth weight of less than 1,000 g, sole diagnosis of hydrocephaly or microencephalopathy, hip subluxation.	Information on inactive waste sites was examined, including air vapor, air particulates, groundwater exposure via wells, and groundwater exposure via basements; exposure was categorized as “high,” “medium,” “low,” or unknown based on probability of exposure; proximity to waste sites was also considered; Most common solvents: TCE, toluene, xylenes, tetrachloroethene, 1,1,1-trichloroethane; Most common metals found lead, mercury, cadmium, chromium, arsenic, and nickel.		Odds ratios (OR), Fisher's exact test, Chi-square, unconditional logistic regression.	13 CNS cases and 351 controls with potential exposures; crude OR: 0.98; When controlling for mother's education, prenatal care, and exposure to a TCE facility, OR was 0.84; CNS and solvents OR: 0.8; CNS and metals OR: 1.0, musculoskeletal defects and solvents OR: 0.9, musculoskeletal defects and pesticides OR: 0.8; higher risk for CNS defects when living close to solvent-emitting facilities.
McCarthy and Jones, 1983	384 industrial workers with solvent poisoning; 103 operated degreasing baths, 62 maintained degreasing baths, 37 used TCE in portable form, 37 misc; no controls.	Individuals poisoned with trichloroethylene, perchloroethylene, and methylchloroform were examined retrospectively; Medical record review; 288 exposed to TCE, 44 to perchloroethylene, 52 to 1,1,1-trichloroethane.	Symptoms reported in occupational/medical records from industrial poisoning incidents; data from 1961 to 1980 on demographics, occupation, work process, type of industry, if incident caused fatality.		17 fatality cases, with 10 in confined spaces; Most common symptoms include effects on CNS; Gastrointestinal and Respiratory symptoms; no strong evidence for cardiac and hepatic toxicity; no change in affected number of workers in 1961 to 1980; greatest effect due to narcotic properties.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Mergler et al., 1991	54 matched pairs; Matching on the basis of age, sex, ethnicity, educational level, sex, and number of children taken from 180 former microelectronics workers exposed to multiple organic solvents and control population of 157 recruited from the same region.	Average duration of employment: 6.1 yrs (range: 1–15 yrs); information about products used and chemical make-up from employer; chemicals: chlorofluorocarbons, chlorinated hydrocarbons, glycol ethers, isopropanol, acetone, toluene, xylene, and ethyl alcohol.	Sociodemographic questionnaire; Monocular examination of visual function: Far visual acuity using a Snellen chart, near visual acuity using a National Optical Visual Chart, color vision using Lanthony D-15, near contrast sensitivity using Vistech grating charts.	Signed-rank Wilcoxon test; Mann-Whitney; Chi-square test for matched pairs; Multiple Regression; Stepwise regression.	Significant difference in near contrast sensitivity: 75% of exposed workers with poorer contrast sensitivity at most frequencies than the matched controls (no difference in results based on smoking, alcohol use, and near visual acuity loss); Significant differences on near visual acuity, color vision, and rates of acquired dyschromatopsia for one eye only; No difference between groups in near or far visual acuity.
Morrow et al., 1989	22 male patients with exposure to multiple organic solvents; 4 involved in litigation; Exclusion: neurologic or psychiatric disorder prior to assessment, alcohol consumption more than 2 drinks/day; Average yrs education 12 (range: 10–16 yrs); average age 38 yrs (range: 27–61); compared to responses of WWII prisoner of war (POW) population with posttraumatic stress disorder (PTSD).	Exposure assessed with questionnaire (duration, type of solvents, weeks since last exposure, cases of excessive exposure); Average exposure duration = 7.3 yrs (range: 2 mos–19 yrs); average weeks since last exposure was 19.8 (range: 1–84 wks); 28% had at least one instance of excessive exposure.	Exposure questionnaire, Group form of the MMPI.	Stepwise multiple regression.	All profiles valid; 90% with at least 2 elevated scales above T score of 70 (clinically significant); Highest elevations on scales 1, 2, 3, and 8; only 1 case within normal limits; when compared to a group of nonpsychiatric patients, exposed patients had more elevations, although both groups have physical complaints; When compared with WWII POW (1/2 diagnosed with PTSD) with similar SES and education, both groups have similar profiles; no age effects found; significant positive correlation between scale 8 and duration of exposure; no significant difference based on time since last exposure or on experiencing excessive exposure.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Morrow et al., 1992	9 men and 3 women occupationally exposed to multiple organic solvents with CNS complaints; all met criteria for mild toxic encephalopathy; exposed group average age was 47 y; Controls: 19 (healthy male volunteers); 26 psychiatric controls (male patients with chronic schizophrenia) average age unexposed controls: 34 yrs; average age schizophrenic patients.: 36 yrs.	Exposure assessed with occupational and environmental exposure questionnaire; mean duration of expo = 3 y (range = <1 d–30 y); average time between last exposure and assessment was 2 y (range; 2 mos–10 y); solvents toluene, TCE.	Auditory event-related potentials under the oddball paradigm: counting and choice reaction time tasks.	Repeated measures ANOVA.	Exposed patients had significant delays in N250 and P300 compared to normal controls and in P300 compared to psychiatric controls; Exposed patients had higher amplitudes for N100, P200, and N250; no difference in P300 amplitude between groups; for the exposed group, P300 positively correlated with exposure duration; findings indicate that solvent exposure affects neural networks.
Seppäläinen and Antti-Poika, 1983	87 patients with solvent poisoning (40 male and 47 female) with occupational exposure to solvents; Follow-up 3–9 yrs after initial diagnosis; Mean age at diagnosis 38.6 (range: 20–59 yrs); no control population.	Chronic exposure with average duration of 10.7 yrs (range:1–33); patients were exposed to TCE ( $n = 21$ ), perchloroethylene ( $n = 12$ ), mixtures of solvents ( $n = 53$ ), mixtures and TCE or perchloroethylene ( $n = 13$ ); Exposure of 54 patients stopped after diagnosis, 33 continued to be exposed; at follow-up, only 5 working with potential of some exposure.	EEG using 10/20 system with 25–30 mins of recording, 3 mins hyperventilation and intermittent photic stimulation; ENMG.	Chi-square, Hypergeometric distribution, McNemar test.	Significantly more ENMG abnormalities at follow-up than at initial diagnosis; Most common finding: slight polyneuropathy; 43% showed improved ENMG, 33% had deteriorated, and 18 pts. with similar ENMG findings (6 normal at both exams); at follow-up, slow-wave abnormalities decreased and paroxysmal abnormalities increased; 41 with improved EEG, 28 with similar EEG (19 had normal EEG at diagnosis), and 18 with deteriorated EEG; EEG pattern of change compared to external head injuries.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Shlomo et al., 2002	Male industrial workers; Mercury exposure group ( <i>n</i> = 40); average age 49.7 (±6.4) yrs; chlorinated hydrocarbons (CHs) exposure group ( <i>n</i> = 37) average age 46.0 (±4.73); Controls, unexposed ( <i>n</i> = 36) average age 49.8 (±5.8), matched by age; (industries not specified).	Interview and record review; Urine samples collected at end of work shift prior to testing and tested for mercury and TCA ; chlorinated hydrocarbons: TCE ( <i>n</i> = 7), PCE ( <i>n</i> = 8), trichloroethane ( <i>n</i> = 22); Mean duration of chloral hydrate (CH) exposure 15.8 (±7.2) yrs; Mean duration of mercury exposure 15.5 (±6.4) yrs; Air sampling: mercury: 0.008 mg/m <sup>3</sup> (TLV = 0.025); TCE: 98 ppm (TLV = 350); PCE: 12.7 ppm (TLV = 25); trichloroethane: 14.4 ppm (TLV = 200); Blood levels: mercury (B-hg) 0.5 gr% (±0.3); TCA urine levels: 1–80% of Biologic Exposure Index (BEI); CH urine levels: 0.11–0.2 of BEI.	Medical history, Neurological tests assessing cranial nerves and cerebellar function; Otoscopy, review of archival data from pure-tone audiometric tests; Auditory brain stem responses (ABR).	Student's t-test, proportions test.	Significant differences between exposed and controls: 33.8% of CH exposed workers with abnormal IPL I-III; 18% of controls; Authors suggest ABRs are sensitive for detecting subclinical CNS effects of CH and mercury.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Till et al., 2001	The children of mothers who had contacted a Canadian pregnancy risk counseling program during pregnancy and reported occupational exposure to solvents ( <i>n</i> = 33); children age range: 3–7; Mothers' occupations: lab technicians, factory workers, graphic designers, artists, and dry cleaning; Controls: 28 matched on age, gender, parental SES, and ethnicity; children of mothers exposed to nonteratogenic agents.	Structured questionnaire about exposure; Method: weight assigned to each exposure Parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low ( <i>n</i> = 19) and high ( <i>n</i> = 14) exposures; solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	NEPSY: Visual Attention, Statue, Tower, Body Part Naming, Verbal Fluency, Speeded Naming, Visuomotor Precision, Imitating Hand Positions, Block Construction, Design Copying, Arrows; Peabody Picture Vocabulary Test; WRAVMA Pegboard test; Child Behavior Checklist (Parent form); Continuous Performance Test.	Mantel Haenszel test, t-test, ANCOVA, Hierarchical multiple linear regression.	Lower composite neurobehavioral scores as exposure increased after adjusting for demographics in Receptive language, expressive language, graphomotor ability; Significantly more exposed children rated with mild-severe problems; No significant difference between groups in attention, visuo-spatial ability, and fine-motor skills; Mean difference on broad- and narrow-band scales of Child Behavior Checklist scores not significant.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Till et al., 2001	Children of mothers who had contacted a Canadian pregnancy risk counseling program during pregnancy and reported occupational exposure to solvents ( <i>n</i> = 32); children age range: 3–7; Mothers’ occupations: lab technicians, factory workers, graphic designers, artists, and dry cleaning; Controls: 27 matched on age, gender, parental SES, and ethnicity; children of mothers exposed to nonteratogenic agents.	Structured questionnaire about exposure; Method: weight assigned to each exposure parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low ( <i>n</i> = 19) and high ( <i>n</i> = 14) exposures; solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	Minimalist test to assess color vision; Cardiff Cards to assess visual acuity.	Independent samples t-tests, Mantel Haenszel Chi test; Wilcoxon-Mann-Whitney test; Kruskal-Wallis Chi square.	Significantly higher number of errors on red-green and blue-yellow discrimination in exposed children compared to controls; exposed children had poorer visual acuity than controls; No significant dose-response relationship between exposure index and color discrimination and visual acuity.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Till et al., 2005	21 infants (9 male, 12 female) of mothers who contacted a Canadian pregnancy risk counseling program and reported occupational exposure to solvents (occupations: factory, lab., dry cleaning; Controls: 27 age-matched infants (17 male, 10 female) of mothers contacted the program due to exposure during pregnancy to nonteratogenic substances).	Structured questionnaire about exposure; Method: weight assigned to each exposure parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low and high exposures; exposure groups: (1) aliphatic and/or aromatic hydrocarbons ( $n = 9$ ), (2) alcohols ( $n = 3$ ), (3) multiple solvents ( $n = 6$ ), (4) PCE, ( $n = 3$ ); mean duration of exposure during pregnancy 27.2 wks. (SD 7.93, range = 12–40); solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	1 <sup>st</sup> visit: Sweep visual evoked potentials (VEP) to assess contrast sensitivity and grating acuity; 2 <sup>nd</sup> visit (2 wks after 1 <sup>st</sup> ): Transient VEPs to assess chromatic and achromatic mechanisms; ophthalmological exam, physical and neurological exam; testers masked to exposure status of infant.	Median split; Multiple Linear Regression; Chi-square, t-test, Mann-Whitney U test, Multivariate ANCOVA, Pearson correlation, Logistic Regression.	Significant decline of contrast sensitivity in low and intermediate spatial frequencies in exposed infants when compared with controls; Significant effect of exposure level on grating acuity, 26.3% of exposed (but 0% of controls) with abnormal VEP to red-green onset stimulus; No differences between groups in latency and amplitude of chromatic and achromatic response.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Valic et al., 1997	138 occupationally exposed and 100 unexposed controls; Exclusion criteria: congenital color vision loss, severe ocular disease, significant vision impairment, tinted glasses or contact lenses, diabetes mellitus, neurological disease, prior severe head or eye injuries, alcohol abuse, medication impairing color vision.	Solvents: TCE, PCE, toluene, xylene; Historical data on duration of exposure protective equipment use, subjective evaluation of exposure, nonoccupational solvent exposure, solvent-related symptoms at work, alcohol and smoking, drug intake; Mean urinary levels of trichloroacetic acid: 1.55 ( $\pm 1.75$ ) mg/L.	Lanthony D15.	Polytomous logistic regression.	Significant effect of age in exposed group; With alcohol of <250 g/wk no significant correlation between color confusion and solvent exposure; Significant interaction between solvent exposure and alcohol intake; Color Confusion Index significantly higher in exposed group with alcohol use of >250 g/wk.
Windham et al., 2006	Children born in 1994 in San Francisco Bay Area with Autism Spectrum Disorders (ASDs) ( $n = 284$ ) and controls ( $n = 657$ ), matched on basis of gender and month of birth.	Birth addresses were geocoded and linked to hazardous air pollutant database; Exposure levels assigned for 19 chemicals; chemicals were grouped based on mechanistic and structural properties; Summary index scores were calculated; risk of ASD calculated in upper quartiles of groups or individual chemical concentrations; Adjustment for demographic factors.	Archival data.	Pearson correlation, Logistic Regression.	Elevated adjusted odds ratios for ASD (by 50%) in top quartile of chlorinated solvents, but not for aromatic solvents; AOR for TCE in 4 <sup>th</sup> quartile = 1.47; lessened when adjusted for metals; correlation between hydrocarbon and metals exposures; when adjusted, increased risk for metals (in 3 <sup>rd</sup> quartile = 1.95; in 4 <sup>th</sup> quartile = 1.7). Contributing compounds: mercury, cadmium, nickel, TCE, vinyl chloride; Results interpreted to suggest relationship between autism and estimated metal and solvent concentrations in air around place of birth residence.



Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Epidemiological Studies: Controlled Exposure Studies; Neurological Effects of Trichloroethylene/Mixed Solvents					
Levy et al., 1981	9 participants (8 males and 1 female) recruited through newspaper ad; 8 h fasting before testing; no control.	Experiment 1: alcohol consumption (3 doses)— blood alcohol levels were measured with breath analyzer pre (multiple baselines) and post test (multiple). Experiment 2: Chloral hydrate administered orally over 2 mins in either 500 mg or 1,500 mg dose; multiple baseline smooth pursuit eye movement (SPEM) tests and multiple posttests after exposure; No control dose administered.	SPEM tests of following a sinusoidally oscillated target at 0.4 Hz; eye movements were recorded through electrodes at each eye.	t-tests; ANOVA.	Experiment 1: prealcohol all subjects had intact SPEM; no significant effect for 1.5 mL/kg of alcohol; significant decline in SPEM at 2.0 and 3.0 mL/kg alcohol; significant dose-effect. Experiment 2: at 500 mg. chloral hydrate, no significant change in pursuit was noted; at 1,500 mg chloral hydrate, qualitative disruptions in pursuit in all participants (4); at 500 mg participants observed to be drowsy; When number reading was added SPEM impairment was 'attenuated' in both alcohol and chloral hydrate conditions.

**Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)**

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Stoppa and McLaughlin, 1967	Chamber study using 2 healthy male volunteers exposed to Freon-113; 1 volunteer exposed to TCE; No control.	Exposure booth was constructed; TCE in air: TCE concentrations: 100, 200, 300, 400 ppm (1965 TLV: 100 ppm for 8-h exposure) in ascending and descending order; total time in chamber: 2.75 h; Freon-113 concentrations: 1,500, 2,500, 3,500, 4,500 ppm (1965 TLV: 1,000 ppm for 8-h exposure), duration 1.5 h; TCE: (1) reduction of weight of compound during exposure was calculated, (2) continuous air sampling in the chamber; Freon-113 in air: (1) and (2) same; (3) gas chromatography on air captured in bottles sealed in the chamber; no control dose given.	Crawford Small Parts Dexterity Test, Necker Cube Test, Card Sorting, Card Sorting with an Auxiliary Task, Dial Display (TCE participant only); Short Employment Test-Clerical (Freon-113 participants only).	Descriptive statistics for air measurement plots by % of TCE change in groups.	No TCE effect at 100 ppm, but test performance deteriorated with increase of TCE concentration; No effect of Freon-113 on psychomotor function at 1,500 ppm, deterioration at 2,500 ppm, as concentration increased, performance deteriorated.

CNS = central nervous system, EEG = electroencephalograph, PCE = perchloroethylene, WHO = World Health Organization.

Table D-3. Literature review of studies of TCE and domains assessed with neurobehavioral/neurological methods

Authors	Year	Study type	Participants no. (N = exposed C = nonexposed)	Dur	PM/RT	VM	Cogn	M&L	M&P	Symp†	Sen††	Resp	Dose effect √ urinary metabolites√	TCE levels
ATSDR	2003	E	N = 116, C = 177	C	ne	ne	ne	ne	ne	ne	A	ne	ne	0 → 23 ppb in dg water
Barret et al.	1984	O	N = 188	C	ne	ne	ne	ne	ne	H, D	T, N, V	ne	√	150 ppm
Barret et al.	1987	O	N = 104, C = 52	C	ne	ne	ne	ne	√	H, D, S, I	T, N	ne	√	ne
Barrett, et al.	1982	O	N = 11, C = 2	C	ne	ne	ne	ne	ne	ne	T	ne	√	ne
Burg, et al.	1995	E	N = 4,281	C	ne	ne	ne	ne	ne	ne	A, N	√	√	ne
Burg and Gist	1999	E	N = 3915	C	ne	ne	ne	ne	ne	ne	A, N	√	√√	4 gps: 2–75,000 ppb
El Ghawabi et al.	1973	O	N = 30, C = 30	C	ne	ne	ne	ne	ne	H, S	(-)	ne	√	165 ppm
Feldman et al.	1988	E	N = 21, C = 27	C	ne	ne	ne	ne	ne	ne	T	ne	ne	ne
Feldman et al.	1992	O	N = 18, C = 30	A,C	ne	ne	ne	ne	ne	ne	T, N	ne	ne	ne
Gamberale, et al.	1976	C	N = 15	A	√	ne	√	(-)	ne	ne	ne	ne	ne	540–1,080 mg <sup>3</sup>
Gash et al.	2007	O	N = 30	C	√	ne	ne	ne	ne	M, N		ne	ne	ne
Grandjean et al.	1955	O	N = 80	C	ne	ne	ne	ne	ne	ne	N	ne	√, √√	6–1,120 ppm
Gun, et al.	1978	O	N = 8, C = 8	C	√	ne	√	ne	ne	ne	N	ne	ne	3–418 ppm
Hirsch, et al.	1996	E	N = 106	C	ne	ne	ne	ne	ne	H	ne	ne	ne	0–2,441 ppb
Kilburn and Thornton	1996	E	N = 237, C = 264	C	√	ne	√	ne	ne	ne	ne	ne	ne	ne
Kilburn and Warshaw	1993	E	N = 544, C = 181	C	√	√	√	√	√	M	T, N	ne	ne	6–500 ppb
Kilburn	2002a	E	N = 236, C = 228	C	ne	ne	√	ne	ne	M	B	ne	ne	6–500 ppb
Kilburn	2002b	E	N = 236, C = 58	C	(-)	ne	ne	ne	(-)	ne	ne	ne	ne	0.2–1,000 ppb
Konietzko, et al.	1975	C	N = 20	A	ne	ne	ne	ne	ne	M	N	ne	√	953 ppm
Kylin, et al. 1967	1967	C	N = 12	A	√	ne	ne	ne	ne	ne	N	ne	ne	1,000 ppm
Landrigan, et al.	1987	O	Residents and 12 W	A,C	ne	ne	√	ne	ne	H, D	ne	ne	√√	≥183,000 ppb
Liu, et al.	1988	O	N = 103, C = 111	C	ne	ne	ne	√	ne	D, N	N	ne	√√	1–100 ppm
Mhiri et al.	2004	O	N = 23, C = 23	A	ne	ne	ne	ne	ne	ne	T	ne	√, √√	ne
Nagaya et al.	1990	O	N = 84, C = 83	C	ne	ne	ne	ne	ne	ne	N	ne	√	22 ppm

**Table D-3. Literature review of studies of TCE and domains assessed with neurobehavioral/neurological methods (continued)**

Authors	Year	Study type	Participants no. (N = exposed C = nonexposed)	Dur	PM/RT	VM	Cogn	M&L	M&P	Symp†	Sen††	Resp	Dose effect √√ urinary metabolites√	TCE levels
Rasmussen and Sabroe	1986	O	N = 240, C = 350	C	ne	ne	ne		√	H,D, I, M	ne	ne	ne	ne
Rasmussen et al.	1993	O	N = 96	C	ne	ne	√	ne	ne	ne	ne	ne	√√	ne
Rasmussen et al.	1993	O	N = 96	C	ne	√	√	ne	ne	ne	ne	ne	√√	ne
Rasmussen et al.	1993	O	N = 99	C	√	ne	ne	ne	ne	ne	N	ne	√√	ne
Reif et al.	2003	E	N = 143	C	√	√	ne	ne	√	M	M	ne	√√	5–15 ppb
Ruijten, et al.	1991	O	N = 31, C = 28	C	√	ne	ne	ne	ne	ne	ne	ne	ne	17–70 ppm
Smith	1970	O	N = 130, C = 63	C	ne	ne	ne	ne	ne	H, D	N	ne	√, √√	ne
Stewart et al	1970	C	N = 13	A	ne	ne	√	ne	ne	H	ne	ne	√	100–202 ppm
Triebig, et al.	1976	C	N = 7, C = 7	A	ne	ne	√	√	√	(-)	ne	ne	√, √√	0–100 ppm
Triebig, et al.	1977	C	N = 7, C = 7	A	ne	ne	√	√	√	M	(-)	ne	√, √√	0–100 ppm
Triebig, et al.	1977	O	N = 8	A,C	ne	√	√	√	ne	ne	ne	ne	√	50 ppm
Triebig, et al.	1982	O	N = 24, C = 24	C	ne	ne	ne	ne	ne	ne	N	ne	√, √√	5–70 ppm
Triebig, et al.	1983	O	N = 66, C = 66	C	ne	ne	ne	ne	ne	N, H	N	ne	√	10–600 mg/m <sup>3</sup>
Troster and Ruff	1990	O	N = 3, C = 60	A	√	√	√	√	√	ne	N	ne	ne	ne
Vernon and Ferguson	1969	C	N = 8	A	√	√	ne	ne	ne	ne	N	ne	√√	0–1000 ppm
Windemuller and Ettema	1978	C	N = 39	A	√	ne	ne	ne	ne	ne	ne	ne	ne	200 ppm
Winneke	1982	O	Not reported	ne	(-)	(-)	ne	ne	ne	ne	ne	ne	ne	50 ppm

†H = Headaches; D = Dizziness; I = Insomnia; S = Sex Probs; M = Mood; N = Neurological.

††A = Audition; B = Balance; V = Vision; T = Trigeminal nerve; N = Other Neurological.

**Study:** C = Chamber; E = Environmental; O = Occupational.

**Duration:** A = Acute, C = Chronic.

√ = positive findings; (-) = findings not significant; ne = not examined or reported; Dur = duration; PM/RT = psychomotor/reaction time; VM = visuo-motor; Cogn = cognitive; M&L = memory and learning; M&P = mood and personality; Symp = symptoms; Sen = sensory; Resp = respiratory.

## **D.2. CENTRAL NERVOUS TOXICITY IN ANIMAL STUDIES FOLLOWING TRICHLOROETHYLENE (TCE) EXPOSURE**

*In vivo* studies in animals and *in vitro* models have convincingly demonstrated that TCE produces functional and physiological neurological changes. Overall, these effects collectively indicate that TCE has central nervous system (CNS) depressant-like effects at lower exposures and causes anesthetic-like effects at high exposures. Studies of TCE toxicity in animals have generally not evaluated whether or not adverse effects seen acutely persist following exposure or whether there are permanent effects of exposure. Exceptions to the focus on acute impairment while under TCE intoxication include studies of hearing impairment and histopathological investigations focused primarily on specific neurochemical pathways, hippocampal development, and demyelination. These persistent TCE effects are discussed initially followed by the results of studies that examined the acute effects of this agent. Summary tables for all the animal studies are at the end of this section.

### **D.2.1. Alterations in Nerve Conduction**

There is little evidence that TCE disrupts trigeminal nerve function in animal studies. Two studies demonstrated TCE produces morphological changes in the trigeminal nerve at a dose of 2,500 mg/kg-day for 10 weeks (Barret et al., 1991, 1992). However, dichloroacetylene, a degradation product formed during the volatilization of TCE was found to produce more severe morphological changes in the trigeminal nerve and at a lower dose of 17 mg/kg-day (Barret et al., 1991, 1992). Only one study (Albee et al., 2006) has evaluated the effects of TCE on trigeminal nerve function, and a subchronic inhalation exposure did not result in any significant functional changes. A summary of these studies is provided in Table D-4.

Barret et al. (1991, 1992) conducted two studies evaluating the effects of both TCE and dichloroacetylene on trigeminal nerve fiber diameter and internodal length as well as several markers for fiber myelination. Female Sprague Dawley rats ( $n = 7/\text{group}$ ) were dosed with 2,500 mg/kg TCE or 17 mg/kg-day dichloroacetylene by gavage for 5 days/week for 10 weeks. These doses were selected based upon the ratio of the LD<sub>50</sub>s (dose at which there is 50% lethality) for these two agents. Two days after administration of the last dose, a morphometric approach was used to study the diameter of teased fibers from the trigeminal nerve. The fibers were classified as Class A or Class B and evaluated for internode length and fiber diameter. TCE-dosed animals only exhibited changes in the smaller Class A fibers where internode length increased marginally (<2%) and fiber diameter increased by 6%. Conversely, dichloroacetylene-treated rats exhibited significant and more robust decreases in internode length and fiber diameter in both fiber classes A and B. Internode length decreased 8% in Class A fibers and 4% in Class B fibers. Fiber diameter decreased 10% in Class A fibers and 6% in Class B fibers.

*This document is a draft for review purposes only and does not constitute Agency policy.*

Biochemical data are presented for fatty acid composition from total lipid extractions from the trigeminal nerve. These two studies identify a clear effect of dichloroacetylene on trigeminal nerve fibers, but the effect by TCE is quite limited.

Albee et al. (2006) evaluated the effects of a subchronic inhalation TCE exposure in Fischer 344 rats (10/sex/group). Rats were exposed to 0-, 250-, 800-, and 2,500-ppm TCE for 6 hours/day, 5 days/week for 13 weeks. At the eleventh week of exposure, rats were surgically implanted with epidural electrodes over the somatosensory and cerebellar regions, and TSEPs were collected 2–3 days following the last exposure. TSEPs were generated using subcutaneous needle electrodes to stimulate the vibrissal pad (area above the nose). The resulting TSEP was measured with electrode previously implanted over the somatosensory region. The TCE exposures were adequate to produce permanent auditory impairment even though TSEPs were unaffected. While TCE appears to be negative in disrupting the trigeminal nerve, the TCE breakdown product, dichloroacetylene, does impair trigeminal nerve function.

Albee et al. (1997) reported that dichloroacetylene disrupted trigeminal nerve somatosensory evoked potentials in Fischer 344 male rats. The subjects were exposed to a mixture of 300-ppm dichloroacetylene, 900-ppm acetylene, and 170-ppm TCE for a single 2.25-hour period. This dichloroacetylene was generated by decomposing TCE in the presence of potassium hydroxide and stabilizing with acetylene. A second treatment group was exposed to a 175-ppm TCE/1,030-ppm acetylene mix with no potassium hydroxide present. Therefore, no dichloroacetylene was present in the second treatment group, providing an opportunity to determine the effects on the trigeminal nerve somatosensory evoked potential in the absence of dichloroacetylene. Evoked potentials from the dichloroacetylene/TCE/acetylene-exposed rats were about 17% smaller measured between peaks I and II and 0.13 msec slower in comparison to the preexposure measurements. Neither latency nor amplitude of this potential changed significantly between the preexposure and postexposure test in the air-exposed animals (control). The dichloroacetylene-mediated evoked potential changes persisted at least until Day 4 postexposure. No changes in evoked potentials were observed in the 175-ppm TCE/1,030-ppm acetylene mix group. It is noteworthy that dichloroacetylene treatment produced broader evidence of toxicity as witnessed by a persistent drop in body weight among subjects over the 7-day postexposure measuring period. In light of the differences observed between the effects of TCE and dichloroacetylene on the trigeminal nerve, it would be instructive to calculate the dose of TCE that would be necessary to produce comparable tissue levels of dichloroacetylene produced in the Albee et al. (1997) study.

Kulig (1987) also measured peripheral (caudal nerve) nerve conduction time in male Wistar rats and failed to show an effect of TCE with exposures as high as 1,500 ppm for 16 hours/day, 5 days/week for 18 weeks.

*This document is a draft for review purposes only and does not constitute Agency policy.*

## **D.2.2. Auditory Effects**

### **D.2.2.1. Inhalation**

The ability of TCE to disrupt auditory function and produce inner ear histopathology abnormalities has been demonstrated in several studies using a variety of test methods. Two different laboratories have identified NOAELs for auditory function of 1,600 ppm following inhalation exposure for 12 hours/day for 13 weeks in Long Evans rats ( $n = 6-10$ ) (Rebert et al., 1991) and 1,500 ppm in Wistar-derived rats ( $n = 12$ ) exposed by inhalation for 18 hours/day, 5 days/week for 3 weeks (Jaspers et al., 1993). The LOAELs identified in these and similar studies are 2,500–4,000-ppm TCE for periods of exposure ranging from 4 hours/day for 5 days to 12 hours/day for 13 weeks (e.g., Muijser et al., 2000; Rebert et al., 1995, 1993; Crofton et al., 1994; Crofton and Zhao, 1997; Fechter et al., 1998; Boyes et al., 2000; Albee et al., 2006). Rebert et al. (1993) estimated acute blood TCE levels associated with permanent hearing impairment at 125  $\mu\text{g/mL}$  by methods that probably underestimated blood TCE values (rats were anaesthetized using 60% carbon dioxide). A summary of these studies is presented in Table D-5.

Rebert et al. (1991) evaluated auditory function in male Long Evans rats ( $n = 10$ ) and F344 rats ( $n = 4-5$ ) by measuring brainstem auditory-evoked responses (BAERs) following stimulation with 4-, 8-, and 16-kHz sounds. The Long-Evans rats were exposed to 0-, 1,600-, or 3,200-ppm TCE, 12 hour/day for 12 weeks and the F344 rats were exposed to 0-, 2,000-, or 3,200-ppm TCE, 12 hours/day for 3 weeks. BAERs were measured every 3 weeks during the exposure and then for an additional 6 weeks following the end of exposure. For the F344 rats, both TCE exposure (2,000 and 3,200 ppm) significantly decreased BAER amplitudes at all frequencies tested. In comparison, Long Evans rats exposed to 3,200-ppm TCE also had significantly decreased BAER amplitude, but exposure to 1,600 ppm did not significantly affect BAERs at any stimulus frequency. These data suggest a LOAEL at 2,000 ppm for the F344 rats and a NOAEL at 1,600 ppm for the Long Evans rats. In subsequent studies, Rebert et al. (1993, 1995) again demonstrated TCE significantly decreases BAER amplitudes and significantly increases the latency of the initial peak (identified as P1).

Jaspers et al. (1993) exposed Wistar-derived WAG-Rii/MBL rats ( $n = 12$ ) to 0, 1,500 and 3,000-ppm TCE exposure for 18 hours/day, 5 days/week for 3 weeks. Auditory function for each frequency was assessed by reflex modification (recording the decibel threshold required to generate a startle response from the rat). Three tones (5, 20, and 35 kHz) were used to test auditory function. The startle measurements were made prior to exposure and at 1, 3, 5, and 6 weeks after exposure. A selective impairment of auditory threshold for animals exposed to 3,000-ppm TCE was observed at all postexposure times at 20 kHz only. No significant effects were noted in rats exposed to 1,500-ppm TCE. This auditory impairment was persistent up through 6 weeks after exposure, which was the last time point presented. There was no

*This document is a draft for review purposes only and does not constitute Agency policy.*

impairment of hearing at either 5 or 25 kHz for animals exposed to 1,500- or 3,000-ppm TCE. This study indicates TCE selectively produces a persistent mid-frequency hearing loss and identifies a NOAEL of 1,500 ppm. Similarly, Crofton et al. (1994) exposed male Long Evans rats ( $n = 7-8$ ) to 3,500-ppm TCE, 8 hours/day for 5 days. Auditory thresholds were determined by reflex modification audiometry 5–8 weeks after exposure. TCE produced a selective impairment of auditory threshold for mid frequency tones, 8 and 16 kHz.

Muijser et al. (2000) evaluated the ability of TCE to potentiate the damaging effect of noise on hearing. Wistar rats ( $n = 8$  per group) were exposed by inhalation to 0 or 3,000-ppm TCE alone for 18 hours/day, 5 days/week for 3 weeks (no noise) or in conjunction with 95-dB broad band noise. The duration of noise exposure is not specified, but presumably was also 18 hours/day, 5 days/week for 3 weeks. Pure tone auditory thresholds were determined using reflex modification audiometry 1 and 2 weeks following the exposures. Significant losses in auditory sensitivity were observed for rats exposed to noise alone at 8, 16, and 20 kHz, for rats exposed to TCE alone at 4, 8, 16, and 20 kHz and for combined exposure subjects at 4, 8, 16, 20, and 24 kHz. The loss of hearing sensitivity at 4 kHz is particularly striking for the combined exposure rats, suggesting a potentiation effect at this frequency. Impairment on this auditory test suggests toxicity at the level of the cochlea or brainstem.

Fechter et al. (1998) exposed Long Evans rats inhalationally to 0 or 4,000-ppm TCE 6 hours/day for 5 days. Three weeks later auditory thresholds were assessed by reflex modification audiometry ( $n = 12$ ), and then 5–7 weeks later, cochlear function was assessed by measuring compound action potentials (CAPs) and the cochlear microphonic response ( $n = 3-10$ ). Cochlear histopathology was assessed at 5–7 weeks ( $n = 4$ ) using light microscopy. Reflex modification thresholds were significantly elevated at 8 and 18 kHz, as were CAP thresholds. The growth of the N1 evoked potential was reduced in the TCE group, and they failed to show normal N1 amplitudes even at supra-threshold tone levels. There was no effect on the sound level required to elicit a cochlear microphonic response of 1  $\mu$ V. Histological data suggest that TCE produces a loss of spiral ganglion cells.

Albee et al. (2006) exposed male and female F344 rats to TCE at 250, 800, or 2,500 ppm for 6 hours/day, 5 days/week, for 13 weeks. At 2,500-ppm TCE, mild frequency-specific hearing deficits were observed, including elevated tone-pip auditory brainstem response thresholds. Focal loss of hair cells in the upper basal turn of the cochlea was observed in 2,500-ppm-exposed rats; this was apparently based upon midmodiolar sections, which lack power in quantification of hair cell death. Except for the cochleas of 2,500-ppm-exposed rats, no treatment-related lesions were noted during the neuro-histopathologic examination. The NOAEL for this study was 800 ppm based on ototoxicity at 2,500 ppm.



The relationship between dose and duration of exposure with respect to producing permanent auditory impairment was presented in Crofton and Zhao (1997) and again in Boyes et al. (2000). The LOAELs identified in Long Evans rats ( $n = 10-12$ ) were 6,000 ppm for a 1-day exposure, 3,200 ppm per day for both the 1- and 4-week exposures, and 2,400 ppm per day for the 13-week exposure. It was estimated from these data that the LOAEL for a 2-year long exposure would be 2,100 ppm. Auditory thresholds were determined for a 16-kHz tone 3–5 weeks after exposure using reflex modification audiometry. Results replicated previous findings of a hearing loss at 16 kHz for all exposure durations. One other conclusion reached by this study is that TCE concentration and not concentration  $\times$  duration of exposure is a better predictor of auditory toxicity. That is, the notion that total exposure represented by the function, concentration (C)  $\times$  time (t), or Haber's law, is not supported. Therefore, higher exposure concentrations for short durations are more likely to produce auditory impairment than are lower concentrations for more protracted durations when total dosage is equated. Thus, consideration needs to be given not only to total C  $\times$  t, but also to peak TCE concentration.

Crofton and Zhao (1997) also presented a benchmark dose for which the calculated dose of TCE would yield a 15-dB loss in auditory threshold. This benchmark response was selected because a 15-dB threshold shift represents a significant loss in threshold sensitivity for humans. The benchmark concentrations for a 15-dB threshold shift are 5,223 ppm for 1 day, 2,108 ppm for 5 days, 1,418 ppm for 20 days, and 1,707 ppm for 65 days of exposure. While more sensitive test methods might be used and other definitions of a benchmark effect chosen with a strong rationale, these data provide useful guidance for exposure concentrations that do yield hearing loss in rats.

These data demonstrate that the ototoxicity of TCE was less than that predicted by a strict concentration  $\times$  time relationship. These data also demonstrate that simple models of extrapolation (i.e.,  $C \times t = k$ , Haber's Law) overestimate the potency of TCE when extrapolating from short-duration to longer-duration exposures. Furthermore, these data suggest that, relative to ambient or occupational exposures, the ototoxicity of TCE in the rat is a high-concentration effect; however, the selection of a 15-dB threshold for detecting auditory impairment along with tests at a single auditory frequency may not capture the most sensitive reliable measure of hearing impairment.

With the exception of a single study performed in the Hartley guinea pig ( $n = 9-10$ ; Yamamura et al., 1983), there are no data in other laboratory animals related to TCE-induced ototoxicity. Yamamura et al. (1983) exposed Hartley guinea pigs to TCE at doses of 6,000, 12,000, and 17,000 ppm for 4 hours/day for 5 days and failed to show an acute impairment of auditory function. However, despite the negative finding in this study, it should be considered that auditory testing was performed in the middle of a laboratory and not in an audiometric sound

*This document is a draft for review purposes only and does not constitute Agency policy.*

attenuating chamber. The influence of extraneous and uncontrolled noise on cochlear electrophysiology is marked and assesses auditory detection thresholds in such an environment unrealistic. Although the study has deficiencies, it is important to note that the guinea pig has been reported to be far less sensitive than the rat to the effects of ototoxic aromatic hydrocarbons such as toluene.

It may be helpful to recognize that the effects of TCE on auditory function in rats are quite comparable to the effects of styrene (e.g., Pryor et al., 1987; Crofton et al., 1994; Campo et al., 2006), toluene (e.g., Pryor et al., 1983; Campo et al., 1999) ethylbenzene (e.g., Cappaert et al., 1999, 2000; Fechter et al., 2007), and *p*-xylene (e.g., Pryor et al., 1987; Gagnaire et al., 2001). All of these aromatic hydrocarbons produce reliable impairment at the peripheral auditory apparatus (inner ear), and this impairment is associated with death of sensory receptor cells, the outer hair cells. In comparing potency of these various agents to produce hearing loss, it appears that TCE is approximately equipotent to toluene and less potent than, in order, ethylbenzene, *p*-xylene, and styrene. Occupational epidemiological studies do appear to identify auditory impairments in workers who are exposed to styrene (Sliwinska-Kowalska et al., 1999; Morioka et al., 2000; Morata et al., 2002) and those exposed to toluene (Abbate et al., 1993; Morata et al., 1997), particularly when noise is also present.

#### **D.2.2.2. Oral and Injection Studies**

No experiments were identified in which auditory function was assessed following TCE administration by either oral or injection routes.

#### **D.2.3. Vestibular System Studies**

The effect of TCE on vestibular function was evaluated by either (1) promoting nystagmus (vestibular system dysfunction) and comparing the level of effort required to achieve nystagmus in the presence and absence of TCE or (2) using an elevated beam apparatus and measuring the balance. Overall, it was found that TCE disrupts vestibular function as presented below. Summary of these studies is found in Table D-6.

Tham et al. (1979, 1984) demonstrated disruption in the stimulated vestibular system in rabbits and Sprague Dawley rats during intravenous (i.v.) infusion with TCE. It is difficult to determine the dosage of TCE necessary to yield acute impairment of vestibular function since testing was performed under continuing infusion of a lipid emulsion containing TCE, and therefore, blood TCE levels were increasing during the course of the study. Tham et al. (1979), for example, infused TCE at doses of 1–5 mg/kg/min reaching arterial blood concentrations as high as 100 ppm. They noted increasing numbers of rabbits experiencing positional nystagmus as blood TCE levels increased. The most sensitive rabbit showed nystagmus at a blood TCE

concentration of about 25 ppm. Similarly, the Sprague Dawley rats also experienced increased nystagmus with a threshold effect level of 120 ppm as measured in arterial blood (Tham et al., 1984). Animals demonstrated a complete recovery in vestibular function when evaluated for nystagmus within 5–10 minutes after the i.v. infusion was stopped.

Niklasson et al. (1993) showed acute impairment of vestibular function in male and female pigmented rats during acute inhalation exposure to TCE (2,700–7,200 ppm) and to trichloroethane (500–2,000 ppm). Both of these agents were able to promote nystagmus during optokinetic stimulation in a dose related manner. While there were no tests performed to assess persistence of these effects, Tham et al. (1979, 1984) did find complete recovery of vestibular function in rabbits ( $n = 19$ ) and female Sprague-Dawley rats ( $n = 11$ ) within minutes of terminating a direct arterial infusion with TCE solution.

The finding that trichloroethylene can yield transient abnormalities in vestibular function is not unique. Similar impairments have been shown for toluene, styrene, along with trichloroethane (Niklasson et al., 1993) and by Tham et al. (1984) for a broad range of aromatic hydrocarbons. The concentration of TCE in blood at which effects were observed for TCE (0.9 mM/L) was quite close to that observed for most of these other vestibulo-active solvents.

#### **D.2.4. Visual Effects**

Changes in visual function have also been demonstrated in animal studies following acute (Boyes et al., 2003, 2005) and subchronic exposure (Blain et al., 1994). Summary of all TCE studies evaluating visual effects in animals can be found in Table D-6. In these studies, the effect of TCE on visual-evoked responses to patterns (Boyes et al., 2003, 2005; Rebert et al., 1991) or a flash stimulus (Rebert et al., 1991; Blain et al., 1994) were evaluated. Overall, the studies demonstrated that exposure to TCE results in significant changes in the visual evoked response, which is reversible once TCE exposure is stopped. Only one study (Rebert et al., 1991) did not demonstrate changes in visual system function with a subchronic TCE exposure, but visual testing was conducted 10 hours after each exposure.

Boyes et al. (2003, 2005) found significant reduction in the visual evoked potential acutely while Long Evans male rats were being exposed to TCE concentrations of 500, 1,000, 2,000, 3,000, 4,000, and 5,000 ppm for intervals ranging from 4 to 0.5 hours, respectively. In both instances, the degree of effect correlated more with brain TCE concentrations than with duration of exposure.

Boyes et al. (2003) exposed adult, male Long-Evans rats to TCE in a head-only exposure chamber while pattern onset/offset visual evoked potentials (VEPs) were recorded. Exposure conditions were designed to provide  $C \times t$  products of 0 ppm/hour (0 ppm for 4 hours) or 4,000 ppm/hour created through four exposure scenarios: 1,000 ppm for 4 hours; 2,000 ppm for

2 hours; 3,000 ppm for 1.3 hours; or 4,000 ppm for 1 hour ( $n = 9-10/\text{concentration}$ ). Blood TCE concentrations were assessed by GC with ECD, and brain TCE concentrations were estimated using a physiologically based pharmacokinetic (PBPK) model. The amplitude of the VEP frequency double component (F2) was decreased significantly ( $p < 0.05$ ) by exposure. The mean amplitude ( $\pm$ SEM in  $\mu\text{V}$ ) of the F2 component in the control and treatment groups measured  $4.4 \pm 0.5$  (0 ppm/4 hours),  $3.1 \pm 0.5$  (1,000 ppm/4 hours),  $3.1 \pm 0.4$  (2,000 ppm/2 hours),  $2.3 \pm 0.3$  (3,000 ppm/1.3 hours), and  $1.9 \pm 0.4$  (4,000 ppm/1 hour). A PBPK model was used to estimate the concentrations of TCE in the brain achieved during each exposure condition. The F2 amplitude of the VEP decreased monotonically as a function of the estimated peak brain concentration but was not related to the area under the curve of the brain TCE concentration. These results indicate that an estimate of the brain TCE concentration at the time of VEP testing predicted the effects of TCE across exposure concentrations and duration.

In a follow-up study, Boyes et al. (2005) exposed Long Evans male rats ( $n = 8-10/\text{concentration}$ ) to TCE exposures of 500 ppm for 4 hours, 1,000 ppm for 4 hours, 2,000 ppm for 2 hours, 3,000 ppm for 1.3 hours, 4,000 ppm for 1 hour and 5,000 ppm for 0.8 hour. VEP recordings were made at multiple time points, and their amplitudes were adjusted in proportion to baseline VEP data for each subject. VEP amplitudes were depressed by TCE exposure during the course of TCE exposure. The degree of VEP depression showed a high correlation with the estimated brain TCE concentration for all levels of atmospheric TCE exposure.

This transient effect of TCE on the peripheral visual system has also been reported by Blain (1994) in which New Zealand albino rabbits were exposed by inhalation to 350- and 700-ppm TCE 4 hours/day, 4 days/week for 12 weeks. Electroretinograms (ERGs) and oscillatory potentials (OPs) were recorded weekly under mesopic conditions. Recordings from the 350- and 700-ppm exposed groups showed a significant increase in the amplitude of the a- and b-waves (ERG). The increase in the a-wave was dose related increasing 30% at the low dose and 84% in the high dose. For the b-wave, the lower exposure dose yielded a larger change from baseline (52%) than did the high dose (33%). The amplitude of the OPs was significantly decreased at 350 ppm (57%) and increased at 700 ppm (117%). The decrease in the oscillatory potentials (OPs) shown in the low-dose group appears to be approximately 25% from 9-12 weeks of exposure. These electroretinal changes were reversed to the baseline value within 6 weeks after the inhalation stopped.

Rebert et al. (1991) evaluated visual evoked potentials (flash evoked potentials and pattern reversal evoked potentials) in male Long Evans rats that received 1,600- or 3,200-ppm TCE for 3 weeks 12 hours/day. No significant changes in flash evoked potential measurements were reported following this exposure paradigm. Limited shifts in pattern reversal visual evoked

*This document is a draft for review purposes only and does not constitute Agency policy.*

potentials were reported during subchronic exposure, namely a reduction in the N1-P1 response amplitude that reached statistical significance following 8, 11, and 14 weeks of exposure. The drop in response amplitude ranged from approximately 20% after 8 weeks to nearly 50% at Week 14. However, this potential recovered completely during the recovery period.

#### **D.2.5. Cognitive Function**

There have been a number of reports (e.g., Kjellstrand et al., 1980; Kulig, 1987; Kishi et al., 1993) showing alteration in performance in learning tasks such as a change in speed to complete the task, but little evidence that learning and memory function are themselves impaired by exposure. Table D-7 presents the study summaries for animal studies evaluating cognitive effects following TCE exposure. Such data are important in efforts to evaluate the functional significance of decreases in myelinated fibers in the hippocampus reported by Isaacson et al. (1990) and disruption of long-term potentiation discovered through *in vitro* testing (Ohta et al., 2001) since the hippocampus has been closely tied to memory formation.

Kjellstrand et al. (1980) exposed Mongolian gerbils ( $n = 12/\text{sex}$ ) to 900-ppm TCE by inhalation for 9 months. Inhalation was continuous except for 1–2 hours/week for cage cleaning. Spatial memory was tested using the radial arm maze task. In this task, the gerbils had to visit each arm of the maze and remember which arm was visited and unvisited in selecting an arm to visit. The gerbils received training and testing in a radial arm maze starting after 2 months of TCE exposure. There was no effect of TCE on learning or performance on the radial arm maze task.

Kishi et al. (1993) acutely exposed Wistar rats to TCE at concentrations of 250, 500, 1,000, 2,000, and 4,000 ppm for 4 hours. Rats were tested on an active (light) signaled shock avoidance operant response. Rats exposed to 250-ppm TCE showed a significant decrease both in the total number of lever presses and in avoidance responses at 140 minutes of exposure compared with controls. The rats did not recover their pre-exposure performance until 140 minutes after the exhaustion of TCE vapor. Exposures in the range 250- to 2,000-ppm TCE for 4 hours produced concentration related decreases in the avoidance response rate. No apparent acceleration of the reaction time was seen during exposure to 1,000- or 2,000-ppm TCE. The latency to a light signal was somewhat prolonged during the exposure to 2,000- to 4,000-ppm TCE. It is estimated that there was depression of the central nervous system with slight performance decrements and the corresponding blood concentration was 40  $\mu\text{g}/\text{mL}$  during exposure. Depression of the central nervous system with anesthetic performance decrements was produced by a blood TCE concentration of about 100  $\mu\text{g}/\text{mL}$ . In general, they observed dose related reductions in total number of lever presses, but these changes may be more

indicative of impaired motor performance than of cognitive impairment. In any event, recovery occurred rapidly once TCE exposure ceased.

Isaacson et al. (1990) studied the effects of oral TCE exposure in weanling rats at exposure doses of 5.5 mg/day for 4 weeks, followed by an additional 2 weeks of exposure at 8.5 mg/day. No significant changes were observed in locomotor activity in comparison to the control animals. This group actually reported improved performance on a Morris swim test of spatial learning as reflected in a decrease in latency to find the platform from 14 seconds in control subjects to 12 seconds in the lower dose TCE group to a latency of 9 seconds in the higher TCE group. The high dose TCE group differed significantly from the control and low TCE dose groups while these latter two groups did not differ significantly from each other. This improvement relative to the control subjects occurred despite a loss in hippocampal myelination, which approached 8% and was shown to be significant using Duncan's multiple range test.

Likewise, Umezu et al. (1997) exposed ICR strain male mice acutely to doses of TCE ranging from 62.5–1,000 mg/kg depending upon the task. They reported a depressed rate of operant responding in a conditioned avoidance task that reached significance with intraperitoneal (i.p.) injections of 1,000 mg/kg. Increased responding during the signaled avoidance period at lower doses (250 and 500 mg/kg) suggests an impairment in ability to inhibit responding or failure to attend to the signal. However, all testing was performed under TCE intoxication.

#### **D.2.6. Psychomotor Effects**

Changes in psychomotor activity such as loss of righting reflex, functional observational battery changes, and locomotor activity have been demonstrated in animals following exposure to TCE. Summaries for some of these studies can be found below and are presented in detail in Table D-8.

##### **D.2.6.1. Loss of Righting Reflex**

Kishi et al. (1993) evaluated the activity and performance of male Wistar rats in a series of tasks following an acute 4-hour exposure to 250, 500, 1,000, 2,000, and 4,000 ppm. They reported disruption in performance at the highest test levels with CNS depression and anesthetic performance decrements. Blood TCE concentrations were about 100 µg/mL in Wistar rats (such blood TCE concentrations were obtained at inhalation exposure levels of 2,000 ppm).

Umezu et al. (1997) studied disruption of the righting reflex following acute injection of 250, 500, 1,000, 2,000, 4000, and 5,000 mg/kg TCE in male ICR mice. At 2,000 mg/kg, loss of righting reflex (LORR) was observed in only 2/10 animals injected. At 4,000 mg/kg, 9/10 animals experienced LORR, and 100% of the animals experienced LORR at 5,000 mg/kg. Shih et al. (2001) reported impaired righting reflexes at exposure doses of 5,000 mg/kg in male

Mfl mic although lower exposure doses were not included. They showed, in addition, that pretreatment prior to TCE with DMSO or disulfiram (which is a CYP2E1 inhibitor) in DMSO could delay loss of the righting reflex in a dose related manner. By contrast, the alcohol dehydrogenase inhibitor, 4-methylpyridine did not delay loss of the righting reflex that resulted from 5,000 mg/kg TCE. These data suggest that the anesthetic properties of TCE involve its oxidation via CYP2E1 to an active metabolite, a finding that is consistent with the anesthetic properties of chloral hydrate.

#### **D.2.6.2. Functional Observational Battery (FOB) and Locomotor Activity Studies**

**D.2.6.2.1. Functional observational battery (FOB) and locomotor activity studies with trichloroethylene (TCE).** A number of papers have measured locomotor activity and used functional observational batteries (FOBs) in order to obtain a more fine grained analysis of the motor behaviors that are impaired by TCE exposure. While exposure to TCE has been shown repeatedly to yield impairments in neuromuscular function acutely, there is very little evidence that the effects persist beyond termination of exposure.

One of the most extensive evaluations of TCE on innate neurobehavior was conducted by Moser et al. (1995, 2003) using FOB testing procedures. Moser et al. (1995) evaluated the effects of acute and subacute (14-day) oral gavage administration of TCE in adult female Fischer 344 rats. Testing was performed both 4 hours post TCE administration and 24 hours after TCE exposure, and a comparison of these two time points along with comparison between the first day and the last day of exposure provides insight into the persistence of effects observed. Various outcome measures were grouped into five domains: autonomic, activity, excitability, neuromuscular, and sensorimotor. Examples of tests included in each of these groupings are as follows: Autonomic—lacrimation, salivation, palpebral closure, pupil response, urination, and defecation; Activity—rearing, motor activity counts home cage position. Excitability—ease of removal, handling reactivity, arousal, clonic, and tonic movements; and Neuromuscular—gait score, righting reflex, fore and hindlimb grip strength, and landing foot splay. Sensorimotor—tail-pinch response, click response, touch response, and approach response. Scoring was performed on a 4-point scale ranging from “1” (normal) to “4” (rare occurrence for control subjects). In the acute exposure, the exposure doses utilized were 150, 500, 1,500, and 5,000 mg/kg TCE in corn oil. These doses represent 3, 10, 30, and 56% of the limit dose. For the 14-day subacute exposure, the doses used were 50, 150, 500, and 1,500 mg/kg. Such doses represent 1, 3, 10, and 30% of the limit dose for TCE.

The main finding for acute TCE administration is that a significant reduction in activity level occurred after the highest dose of TCE (5,000 mg/kg) only. This effect showed substantial recovery 24 hours after exposure though residual decrements in activity were noted.

*This document is a draft for review purposes only and does not constitute Agency policy.*

Neuromuscular function as reflected in the gait score was also severely affected only at 5,000-mg/kg dose and only at the 4-hour test period. Sensorimotor function reflected in response to a sudden click, was abnormal at both 1,500 and 5,000 mg/kg with a slight difference observed at 1,500 mg/kg and a robust difference apparent at 5,000 mg/kg. Additional effects noted, but not shown quantitatively were abnormal home-cage posture, increased landing foot splay, impaired righting and decreased fore and hind limb grip strength. It is uncertain at which doses such effects were observed.

With the exception of sensorimotor function, these same categories were also disrupted in the subacute TCE administration portion of the study. The lack of effect of TCE on sensorimotor function with repeated TCE dosing might reflect either habituation, tolerance, or an unreliable measurement at one of the time points. Given the absence of effect at a range of exposure doses, a true dose-response relationship cannot be developed from these data.

In the subacute study, there are no clearly reliable dose-related differences observed between treated and control subjects. Rearing, a contributor to the activity domain, was elevated in the 500-mg/kg dose group, but was normal in the 1,500-mg/kg group. The neuromuscular domain was noted as significantly affected at 15 days, but it is not clear which subtest was abnormal. It appears that the limited group differences may be random among subjects unrelated to exposure condition.

In a follow-up study, Moser et al. (2003) treated female Fischer 344 rats with TCE by oral gavage for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 mg/kg/d, and testing was undertaken either 4 hours following the first or 10<sup>th</sup> dose as well as 24 hours after these two time points. The authors identified several significant effects produced by TCE administration including a decrease in motor activity, tail pinch responsiveness, reactivity to handling, hind limb grip strength, and body weight. Rats administered TCE also showed significantly more piloerection, higher gait scores, lethality, body weight loss, and lacrimation compared to controls. Only effects observed 4 hours after the 10<sup>th</sup> exposure dose were presented by the authors, and no quantitative information of these measurements is provided.

Albee et al. (2006) exposed male and female Fischer 344 rats to 250-, 800-, and 2,500-ppm TCE for 6 hours/day, 5 days/week for 13 weeks. FOB was performed 4 days prior to exposure and then monthly. Auditory impairments found by others (e.g., Muijser et al., 2000; Rebert et al., 1995; Crofton et al., 1994; Crofton and Zhao, 1997; Fechter et al., 1998; Boyes et al., 2000) were replicated at the highest exposure dose, but treatment related differences in grip strength or landing foot splay were not demonstrated. The authors report slight increases in handling reactivity among female rats and slightly more activity than in controls at an intermediate time point, but apparently did not conduct systematic statistical analyses of these



observations. In any event, there were no statistically significant effects on activity or reactivity by the end of exposure.

Kulig (1987) also failed to show significant effects of TCE inhalation exposure on markers of motor behavior. Wistar rats exposed to 500, 1,000, and 1,500 ppm for 16 hours/day, 5 days/week for 18 weeks failed to show changes in spontaneous activity, grip strength, or coordinated hind limb movement. Measurements were made every three weeks during the exposure period and occurred between 45 minutes and 180 minutes following the previous TCE inhalation exposure. This study establishes a NOAEL of 1,500-ppm TCE with an exposure duration of 16 hours/day.

**D.2.6.2.2. *Acute and subacute oral exposure to dichloroacetic acid on functional observational batteries (FOB).*** Moser et al. (1999) conducted a series of experiments on DCA ranging from acute to chronic exposures. The exposure doses used in the acute experiment were 100, 300, 1,000, and 2,000 mg/kg. In the repeated exposure studies (8 weeks–24 months), doses varied between 16 and 1,000 mg/kg/d. The authors showed pronounced neuromuscular changes in Long Evans and F344 rats dosed orally with the TCE metabolite, DCA, over a period ranging from 9 weeks to 24 months at different exposure doses. Using a multitude of exposure protocols which most commonly entailed daily exposures to DCA either by gavage or drinking water the authors identify effects that were “mostly limited” to the neuromuscular domain. These included disorders of gait, grip strength, foot splay and righting reflex that are dose and duration dependent. Data on gait abnormality and grip strength are presented in greatest detail. In adults exposed to DCA by gavage, gait scores were “somewhat abnormal” at the 7-week test in both the adult Long Evans rats receiving 300 and those receiving 1,000 mg/kg/d. There was no adverse effect in the rats receiving 100 mg/kg/d. In the chronic study, which entailed intake of DCA via drinking water yielding an estimated daily dose of 137 and 235 mg/kg/d “moderately to severely abnormal” gait was observed within 2 months of exposure and dosing was either reduced or discontinued because of the severity of toxicity. For the higher DCA dose, gait scores remained “severely abnormal” at the 24-month test time even though the DCA had been discontinued at the 6-month test time. Hindlimb grip strength was reduced to about ½ the control value in both exposure doses and remained reduced throughout the 24 months of testing even though DCA administration ceased at 6 months for the 235 mg/kg/d group. Forelimb grip strength showed a smaller and apparently reversible effect among DCA treated rats.

### **D.2.6.3. *Locomotor Activity***

Wolff and Siegmund (1978) administered 182 mg/kg TCE (i.p.) in AB mice and observed a decrease in spontaneous locomotor activity. In this study, AB mice were injected

*This document is a draft for review purposes only and does not constitute Agency policy.*

with TCE 30 minutes prior to testing for spontaneous activity at one of 4 time points during a 24 hours/day (0600, 1200, 1800, and 2400 hours). Marked decreases (estimated 60–80% lower than control mice) in locomotor activity were reported in 15-minute test periods. The reduction in locomotion was particularly profound at all time intervals save for the onset of light (0600). Nevertheless, even at this early morning time point, activity was markedly reduced from control levels (60% lower than controls as approximated from a graph).

Moser et al. (1995, 2003) included locomotor activity as one of their measures of neurobehavioral effects of TCE given by gavage over a 10–14 day period. In the 1995 paper, female Fischer 344 rats were dosed either acutely with 150, 500, 1,500 or 5,000 mg/kg TCE or for 14 days with 50, 150, 500 or 1,500 mg/kg. In terms of the locomotor effects, they report that acute exposure produced impaired locomotor scores only at 5,000 mg/kg while in the subacute study, locomotion was impaired at the 500 mg/kg dose, but not at the 1,500 mg/kg dose. In the Moser (2003) study, it appears that 200 mg/kg TCE may actually have increased locomotor activity while the higher test doses (800 and 1,200 mg/kg) decreased activity in a dose related manner. What is common to both studies, however, is a depression in motor activity that occurs acutely following TCE administration and which may speak to the anesthetic if not central nervous system depressive effects of this solvent.

There are also a number of reports (Waseem et al., 2001; Fredriksson et al., 1993; Kulig, 1987) that failed to demonstrate impairment of motor activity or ability following TCE exposure. Waseem et al. (2001) failed to show effects of TCE given in the drinking water of Wistar rats over the course of a 90 day trial. While nominal solvent levels were 350, 700, and 1,400 ppm in the water, no estimate is provided of daily TCE intake or of the stability of the TCE solution over time. However, assuming a daily water intake of 25 mL/day and body weight of 330 g, these exposures would be estimated to be approximately 26, 52, and 105 mg/kg. These doses are far lower than those studied by Moser and colleagues.

Fredriksson et al. (1993) studied the effects of TCE given by oral gavage to male NMRI mice at doses of 50 and 290 mg/kg/d from postnatal Day 10–16 on locomotion assessed either on the day following exposure or at age 60 days. They found no significant effect of TCE on locomotor activity and no consistent effects on other motor behaviors (e.g., rearing).

Waseem et al. (2001) studied locomotor activity in Wistar rats exposed for up to 180 days to 376-ppm TCE by inhalation for 4 hours/day, 5 days/week and acutely intoxicated with TCE. Here the authors report seemingly inconsistent effects of TCE on locomotion. After 30 days of exposure, the treated rats show an increase in locomotor activity relative to control subjects. However, after 60 days of exposure they note a significant *increase* in distance traveled found among experimental subjects, but a decrease in horizontal activity in this experimental group. Moreover, the control subjects vary substantially in horizontal counts among the different time

periods. No differences between the treatment groups are found after 180 days of exposure. It is difficult to understand the apparent discrepancy in results reported at 60 days of exposure.

## **D.2.7. Sleep and Mood Disorders**

### **D.2.7.1. *Effects on Mood: Laboratory Animal Findings***

It is difficult to obtain comparable data of emotionality in laboratory studies. However, Moser et al. (2003) and Albee et al. (2006) both report increases in handling reactivity among rats exposed to TCE. In the Moser study, female Fischer 344 rats received TCE by oral gavage for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 mg/kg/d while Albee et al. (2006) exposed Fischer 344 rats to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm for 6 hours/day, 5 days/week for 13 weeks.

### **D.2.7.2. *Sleep Disturbances***

Arito et al. (1994) exposed male Wistar rats to 50-, 100-, and 300-ppm TCE for 8 hours/day, 5 days/week for 6 weeks and measured electroencephalographic (EEG) responses. EEG responses were used as a measure to determine the number of awake (wakefulness hours) and sleep hours. Exposure to all the TCE levels significantly decreased amount of time spent in wakefulness during the exposure period. Some carry over was observed in the 22-hour postexposure period with significant decreases in wakefulness seen at 100-ppm TCE. Significant changes in wakefulness-sleep elicited by the long-term exposure appeared at lower exposure levels. These data seem to identify a low dose of TCE that has anesthetic properties and established a LOAEL of 50 ppm for sleep changes.

## **D.2.8. Mechanistic Studies**

### **D.2.8.1. *Dopaminergic (DA) Neurons***

In two separate animal studies, subchronic administration of TCE has resulted in a decrease of dopaminergic (DA) cells in both rats and mice. Although the mechanism for DA neurons resulting from TCE exposure is not elucidated, disruption of DA-containing neurons has been extensively studied with respect to Parkinson's Disease and parkinsonism. In addition to Parkinson's Disease, significant study of MPTP and of high-dose manganese toxicity provides strong evidence for extrapyramidal motor dysfunction accompanying loss of dopamine neurons in the substantia nigra. These databases may provide useful comparisons to the highly limited database with regard to TCE and dopamine neuron effects. The studies are presented in Table D-9.

Gash et al. (2007) assessed the effects of subchronic TCE administration on dopaminergic neurons in the central nervous system. Fischer 344 male rats were orally administered by gavage 1,000 mg/kg TCE in olive oil, 5 days/week for 6 weeks. Degenerative

changes in DA containing neurons in the substantia nigra were reported as indexed by a 45% decrease in the number of tyrosine hydroxylase positive cells. Additionally, there was a decrease in the ratio of 3,4-dihydroxyphenylacetic acid, a metabolite of DA, to DA levels in the striatum. This shift in ratio, on the order of 35%, was significant by Student's t-test, suggesting a decrease in release and utilization of this neurotransmitter. While it is possible that long-term adaptation might occur with regard to release rates for DA, the loss of DA cells in the substantia nigra is viewed as a permanent toxic effect. The exposure level used in this study was limited to one high dose and more confidence in the outcome will depend upon replication and development of a dose-response relationship. If the results are replicated, they might be important in understanding mechanisms by which TCE produces neurotoxicity in the central nervous system. The functional significance of such cellular loss has not yet been determined through behavioral testing.

Guehl (1999) also reported persistent effects of TCE exposure on DA neurons. In this study, OF1 male mice ( $n = 10$ ) were injected i.p. daily for 5 days/week for 4 weeks with TCE (400 mg/kg/d). Following a 7 day period when the subjects did not receive TCE, the mice were euthanized and tyrosine hydroxylase immunoreactivity was used to measure neuronal death in the substantia nigra pars compacta. Treated mice presented significant dopaminergic neuronal death (50%) in comparison with control mice based upon total cell counts conducted by an examiner blinded as to treatment group in six samples per subject. The statistical comparison appears to be by Student's t-test (only means, standard deviations, and a probability of  $p < 0.001$  are reported). While this study appears to be consistent with that of Gash et al. (2007) there are some limitations of this study. Specifically, no photomicrographs are provided to assess adequacy of the histopathological material. Additionally, no dose-response data are available to characterize dose-response relationships or identify either a benchmark dose or NOAEL. Behavioral assessment aimed at determining functional significance was not determined.

The importance of these two studies suggesting death of dopaminergic neurons following TCE exposure may be addressable by human health studies because they suggest the potential for TCE to produce a parkinsonian syndrome.

#### **D.2.8.2. *Gamma-Amino Butyric Acid (GABA) and Glutamatergic Neurons***

Disruption of GABAergic and glutamatergic neurons by toxicants can represent serious impairment as gamma-amino butyric acid (GABA) serves as a key inhibitory neurotransmitter while glutamate is equally important as an excitatory neurotoxicant. Moreover, elevations in glutamatergic release have been identified as an important process by which more general neurotoxicity can occur through a process identified as excitotoxicity. The data with regard to

TCE exposure and alteration in GABA and glutamate function is limited. The studies are presented in Table D-10.

Briving et al. (1986) conducted a chronic inhalation exposure in Mongolian gerbils to 50- and 150-ppm TCE continuously for 12 months and reported the changes in amino acid levels in the hippocampus and cerebellar vermis and on high affinity uptake of GABA and glutamate in those same structures. A dose related elevation of glutamine in the hippocampus of approximately 20% at 150 ppm was reported, but no other reliable changes in amino acids in either of these two structures. With regard to high affinity uptake of glutamate and GABA, there were no differences in the hippocampal uptake between control and treated gerbils although in the cerebellar vermis there was a dose related elevation in the high affinity uptake for both of these neurotransmitters. Glutamate uptake was increased about 50% at 50 ppm and 100% at 150 ppm. The corresponding increases for GABA were 69% and 74%. Since control tissue uptake is identified as being 100% rather than as an absolute rate, the ability to assess quality of the control data are limited. It is unclear if this finding in cerebellar vermis is also present in other brain tissues and should be studied further. If these findings are reliable, the changes in high affinity uptake in cerebellum for GABA and glutamate might represent alterations that could have functional outcomes. For example, alteration in GABA release and reuptake from the cerebellum might be consistent with acute alteration in vestibular function described below. However, there are presently no compelling data to support such a relationship.

The change in hippocampal glutamine levels is not readily interpretable. What is not clear from this paper is whether the alterations observed were acute effects observable only while subjects were intoxicated with TCE or whether they would persist once TCE had been removed from the neural tissue. This study used inhalation doses that were at least 1 order of magnitude lower than those required to produce auditory impairment.

A study by Shih et al. (2001) provides indirect evidence in male Mfl mice that TCE exposure by injection might alter GABAergic function. The mice were injected i.p. with 250, 500, 1,000 and 2,000 mg/kg TCE in corn oil and the effect of these treatments on susceptibility to seizure induced by a variety of drugs was observed. Shih et al. report that doses of TCE as low as 250 mg/kg could reduce signs of seizure induced by picrotoxin, bicuculline, and pentylentetrazol. These drugs are all GABAergic antagonists. TCE treatment had a more limited effect on seizure threshold induced by non-GABAergic convulsant drugs such as strychnine (glycine receptor antagonist), 4-aminopyridine (alcohol dehydrogenase inhibitor) and N-methyl-d-aspartate (glutamatergic agonist) than was observed with the GABAergic antagonists. While these data suggest the possibility that TCE could act at least acutely on GABAergic neurons, there are no direct measurements of such an effect. Moreover, there is no obvious relationship between these findings and those of Briving et al. (1986) with regard to

*This document is a draft for review purposes only and does not constitute Agency policy.*

increased high affinity uptake of glutamate and GABA in cerebellum. Beyond that fact, this study does not provide information regarding persistent effects of TCE on either seizure susceptibility or GABAergic function as all measurements were made acutely shortly following a single injection of TCE.

#### **D.2.8.3. Demyelination Following Trichloroethylene (TCE) Exposure**

Because of its anesthetic properties and lipophilicity, it is hypothesized that TCE may disrupt the lipid-rich sheaths that cover many central and peripheral nerves. This issue has also been studied both in specific cranial nerves known to be targets of TCE neurotoxicity (namely the trigeminal nerve) and in the central nervous system including the cerebral cortex, hippocampus and cerebellum in particular. For peripheral and cranial nerves, there are limited nerve conduction velocity studies that are relevant as a functional measure. For central pathways, the most common outcomes studied include histological endpoints and lipid profiles.

A significant difficulty in assessing these studies concerns the permanence or persistence of effect. There is a very large literature unrelated to TCE, which demonstrates the potential for repair of the myelin sheath and at least partial if not full recovery of function. In the studies where nerve myelin markers are assessed, it is not possible to determine if the effects are transient or persistent.

There are two published manuscripts (Isaacson and Taylor, 1989; Isaacson et al., 1990) that document selective hippocampal histopathology when Sprague Dawley rats are exposed to TCE within a developmental model. Both of these studies employed oral TCE administration via the drinking water. In Isaacson and Taylor, (1989), a combined prenatal and neonatal exposure was used while Isaacson's et al. (1990) report focused on a neonatal exposure. In addition, Ohta et al. (2001) presented evidence of altered hippocampal function in an *in vitro* preparation following acute *in vivo* TCE intoxication. The latter most manuscript details a shift in long term potentiation elicited by tetanic shocks to hippocampal slices *in vitro*. In the two developmental studies the exposure doses are expressed in terms of the concentration of TCE placed in the drinking water and the total daily dose is then estimated based upon average water intake by the subjects. However, since the subjects' body weight is not provided, it is not possible to estimate dosage on a mg/kg body weight basis.

Isaacson and Taylor (1989) examined the development of the hippocampus in neonatal rats that were exposed *in utero* and in the preweaning period to TCE via their dam. TCE was added to the drinking water of the dam and daily maternal doses are estimated based upon water intake of the dam as being 4 and 8.1 mg/day. Based upon body weight norms for 70-day old female Sprague Dawley rats, which would predict body weights of about 250 g at that age, such a dose might approach 16–32 mg/kg/d initially during pregnancy. Even if these assumptions

hold true, it is not possible to determine how much TCE was received by the pups although the authors do provide an estimate of fetal exposure expressed as  $\mu\text{g/mL}$  of TCE, trichloroethanol, and trichloroacetic acid. The authors reported a 40% decline in myelinated fibers in the CA1 region of the hippocampus of the weanling rats. There was no effect of TCE treatment on myelination in several other brain regions including the internal capsule, optic tract or fornix and this effect appears to be restricted to the CA1 region of the hippocampus at the tested exposures.

In a second manuscript by that group (Isaacson et al., 1990), weanling rats were exposed to TCE via their drinking water at doses of 5.5 mg/day for 4 weeks or 5.5 mg/day for 4 weeks, a 2 week period with no TCE and then a final 2 weeks of exposure to 8.5 mg/day TCE. Spatial learning was studied using the Morris water maze and hippocampal myelination was examined histologically starting 1 day postexposure. The authors report that the subjects receiving a total of 6 weeks exposure to TCE showed *better* performance in the Morris swim test ( $p < 0.05$ ) than did controls while the 4 week exposed subjects performed at the same level as did controls. Despite this apparent improvement in performance, histological examination of the hippocampus demonstrated a dose dependent relationship with hippocampal myelin being significantly reduced in the TCE exposed groups while normal myelin patterns were found in the internal capsule, optic tract and fornix. The authors did not evaluate the signs of gross toxicity in treated animals such as growth rate, which might have influenced hippocampal development.

Ohta et al. (2001) administered 300 or 1,000 mg/kg TCE, i.p., to male ddY mice. Twenty-four hours after TCE administration, the mice were sacrificed and hippocampal sections were prepared from the excised brains and long term potentiation was measured in the slices. A dose related reduction in the population spike was observed following a tetanic stimulation relative to the size of the population spike elicited in the TCE mice prior to tetany. The spike amplitude was reduced 14% in the 300 mg/kg TCE group and 26% in the 1,000 mg/kg group. Precisely how such a shift in excitability of hippocampal CA1 neurons relates to altered hippocampal function is not certain, but it does demonstrate that injection with 300 mg/kg TCE can have lingering consequences on the hippocampus at least 24 hours following i.p. administration.

A critical area for future study is the potential that TCE might have to produce demyelination in the central nervous system. While it is realistic to imagine that an anesthetic and lipophilic agent such as TCE might interact with lipid membranes and produce alterations, for example, in membrane fluidity at least at anesthetic levels, the data collected by Kyrklund and colleagues suggest that low doses of TCE (50 and 150 ppm chronically for 12 months, 320 ppm for 90 days, 510 ppm 8 hours/day for 5 months) might alter fatty acid metabolism in Sprague Dawley rats and Mongolian gerbils. Because they have not included high doses in their studies and because the low doses produce only sporadic significant effects and these tend to be

*This document is a draft for review purposes only and does not constitute Agency policy.*

of very small magnitude (5–10%) it is not certain that they are truly observing events with biological significance or whether they are observing random effects. A key problem in determining whether the effects under study are spurious or are due to ongoing exposure is that the magnitude and direction of the effect does not grow larger as exposure continues. It could be hypothesized that the alterations in fatty acid metabolism could be an underlying mechanism for demyelination. However, there is not enough evidence to determine if the changes in the lipid profiles lead to demyelination or if the observed effects are purely due to chance. Similarly, the size of statistically significant effects (5–12%) is generally modest. A broad dose-response analysis or the addition of a positive control group that is treated with an agent well-known to produce central demyelination would be important in order to characterize the potency of TCE as an agent that disrupts central nervous system lipid profiles.

Kyrklund and colleagues (e.g., 1986) have generally evaluated the hippocampus, cerebral cortex, cerebellum, and in some instances brainstem in adult gerbil. It is not apparent that one brain region is more vulnerable to the effects of TCE than is another region. While this group does not report significant changes in levels of cholesterol, neutral and acidic phospholipids or total lipid phospholipids, they do suggest a shift in lipid profiles between treated and untreated subjects. Similarly, inhalation exposure to trichloroethane at 1,200 ppm for 30 days (Kyrklund and Haglid, 1991) leads to sporadic changes in fatty acid profiles in Sprague Dawley rats. However, these changes are small and are not always in the same direction as the changes observed following trichloroethylene exposure. In the case of trichloroethane, a NOAEL of 320 ppm for 30 days 24 hours/day was observed and no other doses were evaluated (Kyrklund et al., 1988).

#### **D.2.9. Summary Tables**

Tables D-4 through D-8 summarize the animal studies by neurological domains (Table D-4—trigeminal nerve; Table D-5—ototoxicity; Table D-6—vestibular and visual systems; Table D-7—cognition; and Table D-8—psychomotor function and locomotor activity). For each table, the reference, exposure route, species, dose level, effects and NOAEL/LOAEL are provided. Tables D-9 through D-11 summarize mechanistic (Tables D-9 and D-11) and neurochemical studies (Table D-10). Brief summaries of developmental neurotoxicity studies are provided in Table D-12.



**Table D-4. Summary of mammalian *in vivo* trigeminal nerve studies**

<b>Reference</b>	<b>Exposure route</b>	<b>Species, strain, sex, number</b>	<b>Dose level/ exposure duration</b>	<b>NOAEL: LOAEL</b>	<b>Effects</b>
Barret et al., 1991	Direct Gastric Administration	Rat, Sprague-Dawley, female, 21	0, 2.5 g/kg, acute administration	LOAEL: 2.5 g/kg	Morphometric analysis was used for analyzing the trigeminal nerve. Increase in external and internal fiber diameter as well as myelin thickness was observed in the trigeminal nerve after TCE treatment.
Barret et al., 1992	Direct Gastric Administration	Rat, Sprague-Dawley, female, 18	0, 2.5 g/kg; 1 dose/d, 5 d/wk, 10 wks	LOAEL: 2.5 g/kg	Trigeminal nerve analyzed using morphometric analysis. Increased internode length and fiber diameter in class A fibers of the trigeminal nerve observed with TCE treatment. Changes in fatty acid composition also noted.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL: 2,500 ppm	No effect on trigeminal nerve function was noted at any exposure level.

*This document is a draft for review purposes only and does not constitute Agency policy.*

**Table D-5. Summary of mammalian *in vivo* ototoxicity studies**

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Rebert et al., 1991	Inhalation	Rat, Long Evans, male, 10/group	Long Evans: 0, 1,600, 3,200 ppm; 12 h/d, 12 wk	Long Evans: NOAEL: 1,600 ppm; LOAEL: 3,200 ppm	BAERs were measured. Significant decreases in BAER amplitude and an increase in latency of appearance of the initial peak (P1).
		Rat, F344, male, 4–5/group	F344: 0, 2000, 3200 ppm; 12 h/d, 3 wk	F344: LOAEL: 2,000 ppm	
Rebert et al., 1993		Rat, Long Evans, male, 9/group	0, 2,500, 3,000, 3,500 ppm; 8 h/d, 5 d	NOAEL: 2,500 ppm LOAEL: 3,000 ppm	BAERs were measured 1–2 wk postexposure to assess auditory function. Significant decreases in BAERs were noted with TCE exposure.
Rebert et al., 1995		Rat, Long Evans, male, 9/group	0, 2,800 ppm; 8 h/d, 5 d	LOAEL: 2,800 ppm	BAER measured 2–14 d postexposure at a 16-kHz tone. Hearing loss ranged from 55–85 dB.
Crofton et al., 1994		Rat, Long Evans, male, 7–8/group	0, 3,500 ppm TCE; 8 h/d, 5 d	LOAEL: 3,500 ppm	BAER measured and auditory thresholds determined 5–8 wk postexposure. Selective impairment of auditory function for mid-frequency tones (8 and 16 kHz).
Crofton and Zhao, 1997; Boyes et al., 2000	Inhalation	Rat, Long Evans, male, 9–12/group	0, 4,000, 6,000, 8,000 ppm; 6 h	NOAEL: 6,000 ppm LOAEL: 8,000 ppm	Auditory thresholds as measured by BAERs for the 16-kHz tone increased with TCE exposure.
		Rat, Long Evans, male, 8–10/group	0, 1,600, 2,400, 3,200 ppm; 6 h/d, 5 d	NOAEL: 2,400 ppm LOAEL: 3,200 ppm	
		Rat, Long Evans, male, 8–10/group	0, 800, 1,600, 2,400, 3,200 ppm; 6 h/d, 5 d/wk, 4 wk	NOAEL: 2,400 ppm LOAEL: 3,200 ppm	
		Rat, Long Evans, male, 8–10/group	0, 800, 1,600, 2,400, 3,200 ppm; 6 h/d, 5 d/wk, 13 wk	NOAEL: 1,600 ppm LOAEL: 2,400 ppm	

*This document is a draft for review purposes only and does not constitute Agency policy.*

**Table D-5. Summary of mammalian *in vivo* ototoxicity studies (continued)**

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL*	Effects
Fechter et al., 1998	Inhalation	Rat, Long Evans, male, 12/group	0, 4,000 ppm; 6 h/d, 5 d	LOAEL: 4,000 ppm	Cochlear function measured 5–7 wk after exposure. Loss of spiral ganglion cells noted. Auditory function was significantly decreased as measured by compound action potentials.
Jaspers et al., 1993	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 12/group	0, 1,500, 3,000 ppm; 18 h/d, 5 d/wk, 3 wk	LOAEL: 1,500 ppm	Auditory function assessed repeatedly 1–5 wk postexposure for 5-, 20-, and 35-kHz tones; No effect at 5 or 35 kHz; Decreased auditory sensitivity at 20 kHz.
Muijser et al., 2000	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 8	0, 3,000 ppm	LOAEL: 3,000 ppm	Auditory sensitivity decreased with TCE exposure at 4-, 8-, 16-, and 20-kHz tones.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL: 800 ppm LOAEL: 2,500 ppm	Mild frequency specific hearing deficits; Focal loss of hair cells and cochlear lesions.
Yamamura et al., 1983	Inhalation	Guinea Pig, albino Hartley, male, 7–10/group	0, 6,000, 12,000, 17,000 ppm; 4 h/d, 5 d	NOAEL: 17,000 ppm	No change in auditory sensitivity at any exposure level as measured by cochlear action potentials and microphonics.

*This document is a draft for review purposes only and does not constitute Agency policy.*

**Table D-6. Summary of mammalian sensory studies—vestibular and visual systems**

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
<i>Vestibular system studies</i>					
Tham et al., 1979	Intravenous	Rabbit, strain unknown, sex unspecified, 19	1–5 mg/kg/min	---	Positional nystagmus developed once blood levels reached 30 ppm.
Tham et al., 1984	Intravenous	Rat, Sprague-Dawley, female, 11	80 µg/kg/min	---	Excitatory effects on the vestibule-oculomotor reflex. Threshold effect at blood (TCE) of 120 ppm or 0.9 mM/L.
Niklasson et al., 1993	Inhalation	Rat, strain unknown, male and female, 28	0, 2,700, 4,200, 6,000, 7,200 ppm; 1 h	LOAEL: 2,700 ppm	Increased ability to produce nystagmus.
Umezu et al., 1997	Intraperitoneal	Mouse, ICR, male, 116	0, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 250 mg/kg LOAEL: 500 mg/kg	Decreased equilibrium and coordination as measured by the Bridge test (staying time on an elevated balance beam).
<i>Visual system studies</i>					
Rebert et al., 1991	Inhalation	Rat, Long Evans, male, 10/group	0, 1,600, 3,200 ppm; 12 h/d, 12 weeks	NOAEL: 3,200 ppm	No effect on visual function as measured by visual evoked potential changes.
		Rat, F344, male, 4–5/group	0, 2,000, 3,200 ppm; 12 h/d, 3 wk	NOAEL: 3,200 ppm	
Boyes et al., 2003	Inhalation	Rat, Long Evans, male, 9–10/group	0 ppm, 4 h; 1,000 ppm, 4 h; 2,000 ppm, 2 h; 3,000 ppm, 1.3 h; 4,000 ppm, 1 h	LOAEL: 1,000 ppm, 4 h	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials.
Boyes et al., 2005	Inhalation	Rat, Long Evans, male, 8–10/group	0 ppm, 4 h; 500 ppm, 4 h; 1,000 ppm, 4 h; 2,000 ppm, 2 h; 3,000 ppm, 1.3 h; 4,000 ppm, 1 h; 5,000 ppm, 0.8 h	LOAEL: 500 ppm, 4 h	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials.
Blain et al., 1994	Inhalation	Rabbit, New Zealand albino, male, 6–8/group	0, 350, 700 ppm; 4 h/d, 4 d/wk, 12 wk	LOAEL: 350 ppm	Significant effects noted in visual function as measured by ERG and OPs immediately after exposure. No differences in ERG or OP measurements were noted at 6 wk post-TCE exposure.

*This document is a draft for review purposes only and does not constitute Agency policy.*

**Table D-7. Summary of mammalian cognition studies**

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Kjellstrand et al., 1980	Inhalation	Gerbil, Mongolian, males and females, 12/sex/dose	0, 320 ppm; 9 mos, continuous (24 h/d) except 1–2 h/wk for cage cleaning	NOAEL: 320 ppm	No significant effect on spatial memory (radial arm maze).
Kulig et al., 1987	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, 1,500 ppm; 16 h/d, 5 d/wk, 18 wk	NOAEL: 500 ppm  LOAEL: 1,000 ppm	Increased latency time in the two-choice visual discrimination task (cognitive disruption and/or motor activity related effect).
Isaacson et al., 1990	Oral, drinking water	Rat, Sprague Dawley, male, 12/dose	(1) 0 mg/kg/d, 8 wk (2) 5.5 mg/d (47 mg/kg/d*), 4 wk + 0 mg/kg/d, 4 wk (3) 5.5 mg/d, 4 wk (47 mg/kg/d*) + 0 mg/kg/d, 2 wk + 8.5 mg/d (24 mg/kg/d),* 2 wk	NOAEL: 5.5 mg/d, 4 wk spatial learning  LOAEL: 5.5 mg/d hippocampal demyelination	Decreased latency to find platform in the Morris water maze (Group #3); Hippocampal demyelination observed in all TCE-treated groups.
Kishi et al., 1993	Inhalation	Rats, Wistar, male, number not specified	0, 250,500, 1,000, 2,000, 4,000 ppm, 4 h	LOAEL: 250 ppm	Decreased lever presses and avoidance responses in a shock avoidance task.
Umezu et al., 1997	Intraperitoneal	Mouse, ICR, male, 6 exposed to all treatments	0, 125, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg  LOAEL: 1,000 mg/kg	Decreased response rate in an operant response-cognitive task.
Ohta et al., 2001	Intraperitoneal	Mouse, ddY, male, 5/group	0, 300, 1,000 mg/kg, sacrificed 24 h after injection	LOAEL: 300 mg/kg	Decreased response (LTP response) to tetanic stimulation in the hippocampus.
Oshiro et al., 2004	Inhalation	Rat, Long Evans, male, 24	0, 1,600, 2,400 ppm; 6 h/d, 5 d/wk, 4 wk	NOAEL: 2,400 ppm	No change in reaction time in signal detection task and when challenged with amphetamine, no change in response from control.

\*mg/kg/d conversion estimated from average male Sprague-Dawley rat body weight from ages 21–49 days (118 g) for the 5.5 mg dosing period and ages 63–78 days (354 g) for the 8.5 mg dosing period.

*This document is a draft for review purposes only and does not constitute Agency policy.*

**Table D-8. Summary of mammalian psychomotor function, locomotor activity, and reaction time studies**

Reference	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
Savolainen et al., 1977	Inhalation	Rat, Sprague Dawley, male, 10	0, 200 ppm; 6 h/d, 4 d	LOAEL: 200 ppm	Increased frequency of preening, rearing, and ambulation. Increased preening time.
Wolff and Siegmund, 1978	Intraperitoneal	Mouse, AB, male, 144	0, 182 mg/kg, tested 30 min after injection	LOAEL: 182 mg/kg	Decreased spontaneous motor activity.
Kulig et al., 1987	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, 1,500 ppm; 16 h/d, 5 d/wk, 18 wk	NOAEL: 1,500 ppm	No change in spontaneous activity, grip strength or hindlimb movement.
Motohashi and Miyazaki, 1990	Intraperitoneal	Rat, Wistar, male, 44	0, 1.2 g/kg, tested 30 min after injection	LOAEL: 1.2 g/kg	Increased incidence of rats slipping in the inclined plane test.
			0, 1.2 g/kg/d, 3 d	LOAEL: 1.2 g/kg	Decreased spontaneous motor activity.
Fredericksson et al., 1993	Oral	Mouse, NMRI, male, 12 (3–4 litters)	0, 50, 290 mg/kg/d, at Days 10–16	---	Decreased rearing; No evidence of dose response.
Moser et al., 1995	Oral	Rat, Fischer 344, female, 8/dose	0, 150, 500, 1,500, 5,000 mg/kg, 1 dose	NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg	Decreased motor activity; Neuro-muscular and sensorimotor impairment.
			0, 50, 150, 500, 1,500 mg/kg/d, 14 d	NOAEL: 150 mg/kg/d LOAEL: 500 mg/kg/d	Increased rearing activity.
Bushnell, 1997	Inhalation	Rat, Long Evans, male, 12	0, 400, 800, 1,200, 1,600, 2,000, 2,400 ppm, 1-h/test day, 4 consecutive test days, 2 wk	NOAEL: 800 ppm LOAEL: 1,200 ppm	Decreased sensitivity and increased response time in the signal detection task.

*This document is a draft for review purposes only and does not constitute Agency policy.*

**Table D-8. Summary of mammalian psychomotor function, locomotor activity, and reaction time studies (continued)**

Reference	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL*	Effects
Umezu et al., 1997	Intraperitoneal	Mouse, ICR, male, 6 exposed to all treatments	0, 2,000, 4,000, 5,000 mg/kg – loss of righting reflex measure	LOAEL: 2,000 mg/kg – loss of righting reflex	Loss of righting reflex, decreased operant responses, increased punished responding.
			0, 62.5, 125, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg – operant behavior  NOAEL: 125 mg/kg LOAEL: 250 mg/kg – punished responding	
Bushnell and Oshiro, 2000	Inhalation	Rat, Long Evans, male, 32	0, 2,000, 2,400 ppm; 70 min/d, 9 d	LOAEL: 2,000 ppm	Decreased performance on the signal detection task. Increased response time and decreased response rate.
Nunes et al., 2001	Oral	Rat, Sprague Dawley, male, 10/group	0, 2,000 mg/kg/d, 7 d	LOAEL: 2,000 mg/kg/d	Increased foot splay. No change in any other FOB parameter (e.g., piloerection, activity, reactivity to handling).
Waseem et al., 2001	Oral	Rat, Wistar, male, 8/group	0, 350, 700, 1,400 ppm in drinking water for 90 d	NOAEL: 1,400 ppm	No significant effect on spontaneous locomotor activity.
	Inhalation	Rat, Wistar, male, 6/group	0, 376 ppm for up to 180 d	LOAEL: 376 ppm	Changes in locomotor activity but not consistent when measured over the 180-day period.
Moser et al., 2003	Oral	Rat, Fischer 344, female, 10/group	0, 40, 200, 800, 1,200 mg/kg/d, 10 d	---	Decreased motor activity; Decreased sensitivity; Increased abnormality in gait; Adverse changes in several FOB parameters.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL: 2,500 ppm	No change in any FOB measured parameter.

*This document is a draft for review purposes only and does not constitute Agency policy.*

**Table D-9. Summary of mammalian *in vivo* dopamine neuronal studies**

<b>Reference</b>	<b>Exposure route</b>	<b>Species/strain/sex/number</b>	<b>Dose level/exposure duration</b>	<b>NOAEL; LOAEL</b>	<b>Effects</b>
Guehl et al., 1999	Intraperitoneal administration	Mouse, OF1, male, 10	0, 400 mg/kg	LOAEL: 400 mg/kg	Significant dopaminergic neuronal death in substantia nigra.
Gash et al., 2007	Oral	Rat, Fischer 344, male, 17/group	0, 1,000 mg/kg	LOAEL: 1,000 mg/kg	Degeneration of dopamine-containing neurons in substantia nigra.

*This document is a draft for review purposes only and does not constitute Agency policy.*



**Table D-10. Summary of neurochemical effects with TCE exposure**

Reference	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL	Effects
<i>In vivo studies</i>					
Shih et al., 2001	Intraperitoneal	Mouse, Mfl, male, 6/group	0, 250 500, 1,000, 2,000 mg/kg, 15 min; followed by tail infusion of PTZ (5 mg/mL), picrotoxin (0.8 mg/mL), bicuculline (0.06 mg/mL), strychnine (0.05 mg/mL), 4-AP (2 mg/mL), or NMDA (8 mg/mL)	---	Increased threshold for seizure appearance with TCE pretreatment for all convulsants. Effects strongest on the GABA <sub>A</sub> antagonists, PTZ, picrotoxin, and bicuculline suggesting GABA <sub>A</sub> receptor involvement. NMDA and glycine Rc involvement also suggested.
Briving et al., 1986	Inhalation	Gerbils, Mongolian, male and female, 6/group	0, 50, 150 ppm, continuous, 24 h/d, 12 mos	NOAEL: 50 ppm; LOAEL: 150 ppm for glutamate levels in hippocampus  NOAEL: 150 ppm for glutamate and GABA uptake in hippocampus  LOAEL: 50 ppm for glutamate and GABA uptake in cerebellar vermis	Increased glutamate levels in the hippocampus.  Increased glutamate and GABA uptake in the cerebellar vermis.
Subramoniam et al., 1989	Oral	Rat, Wistar, female,	0, 1,000 mg/kg, 2 or 20 h  0, 1,000 mg/kg/d, 5 d/wk, 1 y	---	PI and PIP2 decreased by 24 and 17% at 2 h.  PI and PIP2 increased by 22 and 38% at 20 h.  PI, PIP, and PIP2 reduced by 52,23, and 45% in 1-yr study.
Kjellstrand et al., 1987	Inhalation	Mouse, NMRI, male	0, 150, 300 ppm, 24 h/d, 4 or 24 d	LOAEL: 150 ppm, 4 and 24 d	Sciatic nerve regeneration was inhibited in both mice and rats.
		Rat, Sprague-Dawley, female	0, 300 ppm, 24 h/d, 4 or 24 d	NOAEL: 300 ppm, 4 d  LOAEL: 300 ppm, 24 d	

*This document is a draft for review purposes only and does not constitute Agency policy.*

**Table D-10. Summary of neurochemical effects with TCE exposure (continued)**

Reference	Exposure route	Species/strain/sex/number	Dose level/exposure duration	NOAEL; LOAEL*	Effects
Haglid et al., 1981	Inhalation	Gerbil, Mogolian, male and female, 6-7/group	0, 60, 320 ppm, 24 h/d, 7 d/wk, 3 mos	LOAEL: 60 ppm, brain protein changes  NOAEL: 60 ppm; LOAEL: 320 ppm, brain DNA changes	(1) Decreases in total brain soluble protein whereas increase in S100 protein. (2) Elevated DNA in cerebellar vermis and sensory motor cortex.

**Table D-11. Summary of *in vitro* ion channel effects with TCE exposure**

Reference	Cellular system	Neuronal channel/receptor	Concentrations	Effects
<i>In vitro studies</i>				
Shafer et al., 2005	PC12 cells	Voltage sensitive calcium channels (VSCC)	0, 500, 1,000, 1,500, 2,000 $\mu$ M	Shift of VSCC activation to a more hyperpolarizing potential. Inhibition of VSCCs at a holding potential of -70 mV.
Beckstead et al., 2000	<i>Xenopus</i> oocytes	Human recombinant Glycine receptor $\alpha$ 1, GABA <sub>A</sub> receptors, $\alpha$ 1 $\beta$ 1, $\alpha$ 1 $\beta$ 2 $\gamma$ 2L	0, 390 $\mu$ M	50% potentiation of the GABA <sub>A</sub> receptors; 100% potentiation of the glycine receptor.
Lopreato et al., 2003	<i>Xenopus</i> oocytes	Human recombinant serotonin 3A receptor	???	Potentiation of serotonin receptor function.
Krasowski and Harrison, 2000	Human embryonic kidney 293 cells	Human recombinant Glycine receptor $\alpha$ 1, GABA <sub>A</sub> receptors $\alpha$ 2 $\beta$ 1	Not provided	Potentiation of glycine receptor function with an EC <sub>50</sub> of 0.65 $\pm$ 0.05 mM. Potentiation of GABA <sub>A</sub> receptor function with an EC <sub>50</sub> of 0.85 $\pm$ 0.2.

EC<sub>50</sub> = median effective concentration.

**Table D-12. Summary of mammalian *in vivo* developmental neurotoxicity studies—oral exposures**

Reference	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL <sup>a</sup>	Effects
Fredriksson et al., 1993	Mouse, NMRI, male pups, 12 pups from 3–4 different litters/group	0, 50, or 290 mg/kg-d PND 10–16	Gavage in a 20% fat emulsion prepared from egg lecithin and peanut oil	Dev. LOAEL: 50 mg/kg/d	Rearing activity sig. ↓ at both dose levels on PND 60.
George et al., 1986	Rat, F334, male and female, 20 pairs/treatment group, 40 controls/sex	0, 0.15, 0.30, or 0.60% microencapsulated TCE.  Breeders exposed 1 wk pre mating, then for 13 wk; pregnant ♀s throughout pregnancy (i.e., 18-wk total).	Dietary	LOAEL: 0.15%	Open field testing in pups: a sig. dose-related trend toward ↑ time required for male and female pups to cross the first grid in the test device.
Isaacson and Taylor, 1989	Rat, Sprague-Dawley, females, 6 dams/group	0, 312, or 625 mg/L (0, 4.0, or 8.1 mg/d) <sup>b</sup>  Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Dev. LOAEL: 312 mg/L	Sig. ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.
Noland-Gerbec et al., 1986	Rat, Sprague-Dawley, females, 9–11 dams/group	0, 312 mg/L (Avg. total intake of dams: 825 mg TCE over 61 d.) <sup>b</sup>  Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Dev. LOEL: 312 mg/L	Sig. ↓ uptake of <sup>3</sup> H-2-DG in whole brains and cerebella (no effect in hippocampus) of exposed pups at 7, 11, and 16 d, but returned to control levels by 21 d.
Taylor et al., 1985	Rat, Sprague-Dawley, females, no. dams/group not reported	0, 312, 625, and 1,250 mg/L  Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Dev. LOAEL: 312 mg/L	Exploratory behavior sig. ↑ in 60- and 90-d old male rats at all treatment levels. Locomotor activity was higher in rats from dams exposed to 1,250-ppm TCE.

<sup>a</sup>NOAEL, LOAEL, and LOEL (lowest-observed-effect level) are based upon reported study findings.

<sup>b</sup>Dose conversions provided by study author(s).

PND = postnatal day.

*This document is a draft for review purposes only and does not constitute Agency policy.*

### D.3. REFERENCES

- Abbate C, Giorgianni C, Munaò F, Brecciaroli R (1993). Neurotoxicity induced by exposure to toluene. An electrophysiologic study. *Int Arch Occup Environ Health*. 64(6):389-392.
- Albee, RR; Nitschke, KD; Mattsson, JL; et al. (1997) Dichloroacetylene: Effects on the rat trigeminal nerve somatosensory evoked potential. *Neurotoxicology and Teratology* 19(1):27-37.
- Albee, RR; Spencer, PJ; Johnson, KA; et al. (2006) Lack of trigeminal nerve toxicity in rats exposed to trichloroethylene vapor for 13 weeks. *Int J Toxicol* 25(6):531-540.
- Albers JW, Wald JJ, Werner RA, Franzblau A, Berent S (1999). Absence of polyneuropathy among workers previously diagnosed with solvent-induced toxic encephalopathy. *J Occup Environ Med*. 41(6):500-509.
- Antti-Poika M (1982). Overall prognosis of patients with diagnosed chronic organic solvent intoxication. *Int Arch Occup Environ Health*. 51(2):127-138.
- Aratani J, Suzuki H, Hashimoto K (1993). Measurement of vibratory perception threshold (VPT) in workers exposed to organic solvents. *Environ Res*. 61(2):357-361.
- Arito, H; Takahashi, M; Ishikawa, T. (1994) Effect of subchronic inhalation exposure to low-level trichloroethylene on heart rate and wakefulness-sleep in freely moving rats. *Jpn J Ind Health (Sangyo Igaku)* 36(1):1-8.
- ATSDR (Agency for Toxic Substances and Disease Registry). (2003) Impact of trichloroethylene exposure on oral motor, speech, and hearing in children. U.S. Department of Health and Human Services, Atlanta, GA.
- Barret, L; Arsac, P; Vincent, M; et al. (1982) Evoked trigeminal nerve potential in chronic trichloroethylene intoxication. *J Toxicol Clin Toxicol* 19(4):419-423.
- Barret, L; Faure, J; Guillard, B; et al. (1984) Trichloroethylene occupational exposure: elements for better prevention. *Int Arch Occup Environ Health* 53(4):283-289.
- Barret, L; Garrel, S; Danel, V; et al. (1987) Chronic trichloroethylene intoxication: a new approach by trigeminal-evoked potentials? *Arch Environ Health* 42(5):297-302.
- Barret, L; Torch, S; Leray, CL; Sarlieve, L; Saxod, R (1992) Morphometric and Biochemical Studies in Trigeminal Nerves of Rat after Trichloroethylene or Dichloroacetylene Oral Administration. *Neurotoxicology* 13:601-614.
- Barret, L; Torch, S; Usson, Y; Barret, L; Torch, S; Usson, Y; et al. (1991) A morphometric evaluation of the effects of trichloroethylene and dichloroacetylene on the rat mental nerve. Preliminary results. *Neurosci Lett* 131(2):141-144.
- Beckstead MJ, Weiner JL, Eger EI 2nd, Gong DH, Mihic SJ (2000). Glycine and gamma-aminobutyric acid(A) receptor function is enhanced by inhaled drugs of abuse. *Mol Pharmacol*. 57(6):1199-1205.
- Binaschi S, and Cantu L. (1982) Vestibular Function In Solvent Exposure: Clinical Criteria. *Neurobehavioral Methods in Occupational Health*. 46: 205-210.
- Blain, L; Lachapelle, P; Molotchnikoff, S. (1994) Electroretinal responses are modified by chronic exposure to trichloroethylene. *Neurotoxicology* 15(3):627-631.
- Bowler RM, Mergler D, Huel G, Harrison R, Cone J (1991). Neuropsychological impairment among former microelectronics workers. *Neurotoxicology*. 12(1):87-103.

*This document is a draft for review purposes only and does not constitute Agency policy.*

- Boyes, WK; Bercegeay, M; Ali, JS; et al. (2003) Dose-based duration adjustments for the effects of inhaled trichloroethylene on rat visual function. *Toxicol Sci* 76(1):121–130.
- Boyes, WK; Bercegeay, M; Krantz, T; et al. (2005) Momentary brain concentration of trichloroethylene predicts the effects on rat visual function. *Toxicol Sci* 87(1):187–196.
- Boyes, WK; Bushnell, PJ; Crofton, KM; et al. (2000) Neurotoxic and pharmacokinetic responses to trichloroethylene as a function of exposure scenario. *Environ Health Perspect* 108(Suppl 2):317–322.
- Briving, C; Jacobson, I; Hamberger, A; et al. (1986) Chronic effects of perchloroethylene and trichloroethylene on the gerbil brain amino acids and glutathione. *Neurotoxicology* 7(1):101–108.
- Burg, JR; Gist, G; Allred, SL; et al. (1995) The national exposure registry - morbidity analyses of noncancer outcomes from the trichloroethylene subregistry baseline data. *Int J Occ Med Toxicol* 4(2):237–257.
- Burg, JR; Gist, GL. (1999) Health effects of environmental contaminant exposure: an intrafile comparison of the Trichloroethylene Subregistry. *Arch Environ Health* 54(4):231–241.
- Bushnell PJ (1997). Concentration-time relationships for the effects of inhaled trichloroethylene on signal detection behavior in rats. *Fundam Appl Toxicol*. 36(1):30-38.
- Bushnell PJ, Oshiro WM (2000). Behavioral components of tolerance to repeated inhalation of trichloroethylene (TCE) in rats. *Neurotoxicol Teratol*. 22(2):221-229.
- Buxton, P.H., and Hayward, M. (1967). Polyneuritis cranialis associated with industrial trichloroethylene poisoning. *J. Neurol. Neurosurg. Psych.* 30, 511.
- Campo P, Blancher V, Payan JP, Cossec B, Ducos P. Toluene toxicokinetics and metabolism parameters in the rat and guinea pig. *Environmental Toxicology and Pharmacology* 21 (2006) 276–282.
- Campo P, Loquet G, Blachère V, Roure M (1999). Toluene and styrene intoxication route in the rat cochlea. *Neurotoxicol Teratol*. 21(4):427-434.
- Cappaert NL, Klis SF, Baretta AB, Muijser H, Smoorenburg GF. Ethyl benzene-induced ototoxicity in rats: a dose-dependent mid-frequency hearing loss. *J Assoc Res Otolaryngol*. 2000 Dec;1(4):292–9.
- Cappaert NL, Klis SF, Muijser H, de Groot JC, Kulig BM, Smoorenburg GF. The ototoxic effects of ethyl benzene in rats. *Hear Res*. 1999 Nov;137(1–2):91–102.
- Chalupa, B., J. Synkova, and M. Sevcik 1960. The Assessment of Electro-encephalographic changes in memory disturbances in acute intoxications with industrial poisons, *Brit. J. Ind. Med.* 17, 238.
- Colvin M, Myers J, Nell V, Rees D, Cronje R (1993). A cross-sectional survey of neurobehavioral effects of chronic solvent exposure on workers in a paint manufacturing plant. *Environ Res*. 63(1):122-132.
- Crofton, KM Lassiter, TL and Rebert, CS. Solvent-induced ototoxicity in rats: an atypical selective mid-frequency hearing deficit, *Hear. Res.*, 80 (1994, 25–30.
- Crofton, KM; Zhao, X. (1997) The ototoxicity of trichloroethylene: extrapolation and relevance of high-concentration, short-duration animal exposure data. *Fundam Appl Toxicol* 38(1):101–106.
- Daniell WE, Claypoole KH, Checkoway H, Smith-Weller T, Dager SR, Townes BD, Rosenstock L (1999). Neuropsychological function in retired workers with previous long-term occupational exposure to solvents. *Occup Environ Med*. 56(2):93-105.

*This document is a draft for review purposes only and does not constitute Agency policy.*

Donoghue AM, Dryson EW, Wynn-Williams G (1995). Contrast sensitivity in organic-solvent-induced chronic toxic encephalopathy. *J Occup Environ Med.* 37(12):1357-1363

El Ghawabi, SM; Mansoor, MB; El Gamel, MS; et al. (1973) Chronic trichloroethylene exposure. *J Egypt Med Assoc* 56(11–12):715–724.

Elofsson SA, Gamberale F, Hindmarsh T, Iregren A, Isaksson A, Johnsson I, Knave B, Lydahl E, Mindus P, Persson HE, Philipson B, Steby M, Struwe G, Söderman E, Wennberg A, Widén L (1980). Exposure to organic solvents. A cross-sectional epidemiologic investigation on occupationally exposed care and industrial spray painters with special reference to the nervous system. *Scand J Work Environ Health.* 6(4):239-273.

Fechter LD, Gearhart C, Fulton S, Campbell J, Fisher J, Na K, Cocker D, Nelson-Miller A, Moon P, Pouyatos B. Promotion of noise-induced cochlear injury by toluene and ethylbenzene in the rat. *Toxicol Sci.* 2007 Aug;98(2):542–51.

Fechter, LD, Liu, Y., Herr, DW, Crofton, KM. Trichloroethylene ototoxicity: Evidence for a cochlear origin., *Toxicol. Sci.* 42, 28–35, 1998.

Feldman, RG; Chirico-Post, J; Proctor, SP. (1988) Blink reflex latency after exposure to trichloroethylene in well water. *Arch Environ Health* 43(2):143–148.

Feldman, RG; Niles, C; Proctor, SP; et al. (1992) Blink reflex measurement of effects of trichloroethylene exposure on the trigeminal nerve. *Muscle Nerve* 15(4):490–495.

Fredriksson et al., *tox lett.* 66, 1993, 13–19. Altered behavior in adult mice orally exposed to tri- and tetrachloroethylene as neonates.

Gagnaire F, Marignac B, Langlais C, Bonnet P (2001). Ototoxicity in rats exposed to ortho-, meta- and paraxylene vapours for 13 weeks. *Pharmacol Toxicol.* 89(1):6-14.

Gamberale, F; Annwall, G; Olson, BA. (1976) Exposure to trichloroethylene III. Psychological functions. *Scand J Work Environ Health* 2(4):220–224.

Gash, DM; Rutland, K; Hudson, NL; et al. (2007) Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity. *Ann Neurol.*

George, JD; Reel, JR; Myers, CB; Lawton, AD; Lamb, JC. (1986) Trichloroethylene: reproduction and fertility assessment in F344 rats when administered in the feed. RTI Project No. 310-2344, NTP-86-085. National Institute of Environmental Health Sciences, National Toxicology Program, RTP, NC.

Grandjean, E; Munchinger, R; Turrian, V; et al. (1955) Investigations into the effects of exposure to trichloroethylene in mechanical engineering. *Br J Ind Med* 12(2):131–142.

Gregersen P. (1988) Neurotoxic effects of organic solvents in exposed workers: two controlled follow-up studies after 5.5 and 10.6 years. *Am J Ind Med.* 14(6):681-701.

Guehl, D; Bezar, E; Dovero, S; et al. (1999) Trichloroethylene and parkinsonism: a human and experimental observation. *Eur J Neurol* 6(5):609–611.

Gun, RT; Grygorcewicz, C; Nettelbeck, TJ. (1978) Choice reaction time in workers using trichloroethylene. *Med J Aust* 1(10):535–536.

Haglid KG, Briving C, Hansson HA, Rosengren L, Kjellstrand P, Stavron D, Swedin U, Wronski A (1981). Trichloroethylene: long-lasting changes in the brain after rehabilitation. *Neurotoxicology.* 2(4):659-673.

*This document is a draft for review purposes only and does not constitute Agency policy.*

- Hirsch, AR; Rankin, KM; Panelli, PP. (1996) Trichloroethylene exposure and headache. *Headache Quarterly* 7(2):126–138.
- Isaacson, LG; Spohler, SA; Taylor, DH. (1990) Trichloroethylene affects learning and decreases myelin in the rat hippocampus. *Neurotoxicol Teratol* 12(4):375–381.
- Isaacson, LG; Taylor, DH. (1989) Maternal exposure to 1,1,2-trichloroethylene affects myelin in the hippocampal formation of the developing rat. *Brain Res* 488(1–2):403–407.
- Jaspers, RMA, Muijser, H., et al., Mid-frequency hearing loss and reduction of acoustic startle responding in rats following trichloroethylene exposure. *Neurotoxicol. Teratol.*, 15, 407–412, 1993.
- Juntunen J, Antti-Poika M, Tola S, Partanen T (1982). Clinical prognosis of patients with diagnosed chronic solvent intoxication. *Acta Neurol Scand.* 65(5):488-503.
- Juntunen J, Hupli V, Hernberg S, Luisto M (1980). Neurological picture of organic solvent poisoning in industry. A retrospective clinical study of 37 patients. *Int Arch Occup Environ Health.* 46(3):219-331.
- Kilburn, KH. (2002a): Is neurotoxicity associated with environmental trichloroethylene (TCE)? *Arch Environ Health* 57(2):113–120.
- Kilburn, KH. (2002b): Do duration of exposure, proximity to electronic manufacturing plants, and involvement in a lawsuit affect chlorinated solvent toxicity? *Arch Environ Health* 57(2):121–126
- Kilburn, KH; Thornton, JC. (1996) Prediction equations for simple and visual two-choice reactions times in environmental neurotoxicology. *Arch Environ Health* 51(6):439–444.
- Kilburn, KH; Warshaw, RH. (1993) Effects on neurobehavioral performance of chronic exposure to chemically contaminated well water. *Toxicol Ind Health* 9(3):391–404.
- Kishi, R; Harabuchi, I; Ikeda, T; et al. (1993) Acute effects of trichloroethylene on blood concentrations and performance decrements in rats and their relevance to humans. *Br J Ind Med* 50(5):470–480.
- Kjellstrand, P; Kanje, M; Bjerkemo, M. (1987) Regeneration of the sciatic nerve in mice and rats exposed to trichloroethylene. *Toxicol Lett* 38(1–2):187–191.
- Kjellstrand, P; Lanke, J; Bjerkemo, M; et al. (1980) Irreversible effects of trichloroethylene exposure on the central nervous system. *Scand J Work Environ Health* 6(1):40–47.
- Konietzko, H; Elster, I; Sayer, H; et al.,(1975) EEG Changes Under Standardized Trichloroethylene Load; *Int. Arch. Occup. Environ. Hlth* 35, 257–264 <Original> Zentralnervose schaden durch trichlorathylen. *Staub, Reinhaltung Der Luft* 35(6):240–241.
- Krasowski MD, Harrison NL. 2000. The actions of ether, alcohol and alkane general anaesthetics on GABAA and glycine receptors and the effects of TM2 and TM3 mutations. *Br J Pharmacol.* 129(4):731–43.
- Kulig, BM. (1987) The effects of chronic trichloroethylene exposure on neurobehavioral functioning in the rat. *Neurotoxicol Teratol* 9(2):171–178.
- Kylin, B; Axell, K; Samuel, HE; et al. (1967) Effect Of Inhaled Trichloroethylene On The CNS As Measured By Optokinetic Nystagmus. *Archives of Environmental Health* 15:48–52.
- Kyrklund T, Haglid K (1991). Brain lipid composition in guinea pigs after intrauterine exposure to perchloroethylene. *Pharmacol Toxicol.* 68(2):146-148.

*This document is a draft for review purposes only and does not constitute Agency policy.*



- Kyrklund T, Kjellstrand P, Haglid KG (1986). Fatty acid changes in rat brain ethanolamine phosphoglycerides during and following chronic exposure to trichloroethylene. *Toxicol Appl Pharmacol.* 85(2):145-153.
- Kyrklund T, Kjellstrand P, Haglid KG (1988). Effects of exposure to Freon 11, 1,1,1-trichloroethane or perchloroethylene on the lipid and fatty-acid composition of rat cerebral cortex. *Scand J Work Environ Health.* 14(2):91-94.
- Landrigan, PJ; Kominsky, JR; Stein, GF; et al. (1987) Common-source community and industrial exposure to trichloroethylene. *Arch Environ Health* 42(6):327–332.
- Laslo-Baker D, Barrera M, Knittel-Keren D, Kozer E, Wolpin J, Khattak S, Hackman R, Rovet J, Koren G (2004). Child neurodevelopmental outcome and maternal occupational exposure to solvents. *Arch Pediatr Adolesc Med.* 158(10):956-961.
- Lee DH, Park IG, Kim JH, Lee YH, Kim D, Kang SK (1998). Neurobehavioral changes in shoe manufacturing workers. *Neurotoxicol Teratol.* 20(3):259-263.
- Levy DL, Lipton RB, Holzman PS (1981). Smooth pursuit eye movements: effects of alcohol and chloral hydrate. *J Psychiatr Res.* 16(1):1-11.
- Lindström K (1973). Psychological performances of workers exposed to various solvents. *Work Environ Health.* 10:150-155.
- Lindström K (1980). Changes in psychological performances of solvent-poisoned and solvent-exposed workers. *Am J Ind Med.* 1(1):69-84.
- Lindström K, Antti-Poika M, Tola S, Hyytiäinen A (1982). Psychological prognosis of diagnosed chronic organic solvent intoxication. *Neurobehav Toxicol Teratol.* 4(5):581-588.
- Liu, YT; Jin, C; Chen, Z; et al. (1988) Increased subjective symptom prevalence among workers exposed to trichloroethylene at sub-OEL levels. *Tohoku J Exp Med* 155(2):183–195.
- Lopreato GF, Phelan R, Borghese CM, Beckstead MJ, Mihic SJ. (2003) Inhaled drugs of abuse enhance serotonin-3 receptor function. *Drug Alcohol Depend.* 70(1):11–5.
- Marshall EG, Gensburg LJ, Deres DA, Geary NS, Cayo MR (1997). Maternal residential exposure to hazardous wastes and risk of central nervous system and musculoskeletal birth defects. *Arch Environ Health.* 52(6):416-425.
- McCarthy TB, Jones RD (1983). Industrial gassing poisonings due to trichlorethylene, perchlorethylene, and 1-1-1 trichloroethane, 1961-80. *Br J Ind Med.* 40(4):450-455.
- McCunney, R.J., Diverse manifestations of trichloroethylene (1988). *Br J Ind Med* 45:122–126.
- Mergler D, Huel G, Bowler R, Frenette B, Cone J (1991). Visual dysfunction among former microelectronics assembly workers. *Arch Environ Health.* 46(6):326-334.
- Mhiri, C; Choyakh, F; Ben, HM; et al. (2004) Trigeminal somatosensory evoked potentials in trichloroethylene-exposed workers. *Neurosciences* 9(2):102–107.
- Mitchell, A. B. S., and Parsons-Smith, B. G., (1969) Trichloroethylene Neuropathy. *Brit. med. J.* 1, 422–423
- Morata TC, Fiorini AC, Fischer FM, Colacioppo S, Wallingford KM, Krieg EF, Dunn DE, Gozzoli L, Padrão MA, Cesar CL (1997). Toluene-induced hearing loss among rotogravure printing workers. *Scand J Work Environ Health.* 23(4):289-298.

*This document is a draft for review purposes only and does not constitute Agency policy.*

- Morata TC, Johnson AC, Nysten P, Svensson EB, Cheng J, Krieg EF, Lindblad AC, Ernstgård L, Franks J (2002). Audiometric findings in workers exposed to low levels of styrene and noise. *J Occup Environ Med.* 44(9):806-814.
- Morioka I, Miyai N, Yamamoto H, Miyashita K (2000). Evaluation of combined effect of organic solvents and noise by the upper limit of hearing. *Ind Health.* 38(2):252-257.
- Morrow LA, Ryan CM, Goldstein G, Hodgson MJ (1989). A distinct pattern of personality disturbance following exposure to mixtures of organic solvents. *J Occup Med.* 31(9):743-746.
- Morrow LA, Steinhauer SR, Hodgson MJ (1992). Delay in P300 latency in patients with organic solvent exposure. *Arch Neurol.* 49(3):315-320.
- Moser VC, Phillips PM, McDaniel KL, MacPhail RC (1999). Behavioral evaluation of the neurotoxicity produced by dichloroacetic acid in rats. *Neurotoxicol Teratol.* 21(6):719-731.
- Moser, Cheek and MacPhail. A multidisciplinary approach to toxicological screening III. Neurobehavioral toxicity. *J Toxicol. Environ. Hlth.*, 1995, 45, 173–210.
- Moser, VC; MacPhail, RC; Gennings, C. (2003) Neurobehavioral evaluations of mixtures of trichloroethylene, heptachlor, and di(2-ethylhexyl)phthalate in a full-factorial design. *Toxicology* 188(2–3):125–137.
- Motohashi Y, Miyazaki Y (1990). Time-dependent effects of trichloroethylene on motor activity in rats. *Chronobiol Int.* 7(3):193-201.
- Muijser, H; Lammers, J; Kulig, BM. (2000) Effects of exposure to trichloroethylene and noise on hearing in rats. *Noise and Health* 6:57–66.
- Nagaya, T; Ishikawa, N; Hata, H. (1990) No Change in Serum Dopamine-beta-hydroxylase Activity in Workers Exposed to Trichloroethylene. *Toxicology Letters* 54(2/3):221–227.
- Niklasson, Tham, Larsby, and Eriksson, Effects of toluene, styrene, trichloroethylene and trichloroethane on the vestibule and opto-oculo motor system in rats, *Neurotoxicol. Teratol.*, 1993, 15, 327–334.
- Noland-Gerbec, Pfohl, Taylor and Bull. 2-deoxyglucose uptake in the developing rat brain upon pre and postnatal exposure to trichloroethylene, *Neurotoxicology*, 7,, 157–164, 1986.
- Nunes J, Ehrlich M, Robertson J (2001). Toxicosis associated with dual oral exposure of rats to lead and trichloroethylene. *Toxicol Pathol.* 29(4):451-457.
- Ohta, M; Saito, T; Saito, K; et al. (2001) Effect of trichloroethylene on spatiotemporal pattern of LTP in mouse hippocampal slices. *Int J Neurosci* 111(3–4):257–271.
- Oshiro WM, Krantz QT, Bushnell PJ (2004). A search for residual behavioral effects of trichloroethylene (TCE) in rats exposed as young adults. *Neurotoxicol Teratol.* 26(2):239-251.
- Pryor GT, Dickinson J, Howd RA, Rebert CS (1983). Transient cognitive deficits and high-frequency hearing loss in weanling rats exposed to toluene. *Neurobehav Toxicol Teratol.* 5(1):53-57.
- Pryor GT, Rebert CS, Howd RA (1987). Hearing loss in rats caused by inhalation of mixed xylenes and styrene. *J Appl Toxicol.* 7(1):55-61.
- Rasmussen, K; Arlien-Soborg, P; Sabroe, S. (1993c) Clinical neurological findings among metal degreasers exposed to chlorinated solvents. *Acta Neurol Scand* 87(3):200–204.

*This document is a draft for review purposes only and does not constitute Agency policy.*

- Rasmussen, K; Jeppesen, HJ; Sabroe, S. (1993a) Solvent-Induced Chronic Toxic Encephalopathy. *American Journal of Industrial Medicine* 23:779–792.
- Rasmussen, K; Jeppesen, HJ; Sabroe, S. (1993b) Psychometric tests for assessment of brain function after solvent exposure. *Am J Ind Med* 24(5):553–565.
- Rasmussen, K; Sabroe, S. (1986) Neuropsychological symptoms among metal workers exposed to halogenated hydrocarbons. *Scand J Soc Med* 14(3) :161–168.
- Rebert CS, Boyes WK, Pryor GT, Svendsgaard DJ, Kassay KM, Gordon GR, Shinsky N (1993). Combined effects of solvents on the rat's auditory system: styrene and trichloroethylene. *Int J Psychophysiol.* 14(1):49–59
- Rebert CS, Day VL, Matteucci MJ, Pryor GT (1991). Sensory-evoked potentials in rats chronically exposed to trichloroethylene: predominant auditory dysfunction. *Neurotoxicol Teratol.* 13(1):83–90
- Rebert CS, Schwartz RW, Svendsgaard DJ, Pryor GT, Boyes WK (1995). Combined effects of paired solvents on the rat's auditory system. *Toxicology.* 105(2–3):345–54.
- Reif, JS; Burch, JB; Nuckols, JR; et al. (2003) Neurobehavioral effects of exposure to trichloroethylene through a municipal water supply. *Environ Res* 93(3):248–258.
- Ruijten, M; Verberk, MM; Salle, HJ. (1991) Nerve function in workers with long term exposure to trichloroethene. *Br J Ind Med* 48(2):87–92.
- Salvini, M., Binasch, S., and Riva, M. (1971). Evaluation of the psychophysiological functions in humans exposed to trichloroethylene. *Br J Ind Med*, 28:293–295.
- Savolainen H, Pfäffli P, Tengén M, Vainio H. (1977). Trichloroethylene and 1,1,1-trichloroethane: effects on brain and liver after five days intermittent inhalation. *Arch Toxicol.* 38(3):229-237. Available at [http://www.ncbi.nlm.nih.gov/pubmed/578726?ordinalpos=6&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed\\_Resul tsPanel.Pubmed\\_DefaultReportPanel.Pubmed\\_RVDocSum](http://www.ncbi.nlm.nih.gov/pubmed/578726?ordinalpos=6&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_Resul tsPanel.Pubmed_DefaultReportPanel.Pubmed_RVDocSum).
- Seppäläinen AM, Antti-Poika M (1983). Time course of electrophysiological findings for patients with solvent poisoning. A descriptive study. *Scand J Work Environ Health.* 9(1):15-24 (As cited in Winneke, 1982).
- Shafer, TJ; Bushnell, PJ; Benignus, VA; et al. (2005) Perturbation of voltage-sensitive Ca<sup>2+</sup> channel function by volatile organic solvents. *J Pharmacol Exp Ther* 315(3):1109–1118.
- Shih, CL, Chen, HH, Chiu, TH. Acute exposure to trichloroethylene differentially alters the susceptibility to chemoconvulsants in mice. *Toxicology*, 162, 2001, 35–42.
- Shlomo M, Avraham F, Moshe H, Mario S, Jacklin S, Mordechai H (2002). Effects of Occupational Exposure to Mercury or Chlorinated Hydrocarbons on the Auditory Pathway. *Noise and Health.* 4(16): 71-77.
- Sliwińska-Kowalska M, Jedlińska U, Rządzińska A, Rajkowska E (1999). Hair cell regeneration in a chicken's inner ear after damage due to exposure to industrial noise *Otolaryngol Pol.* 53(4):469-477.
- Smith, GF. (1970) The investigation of the mental effects of trichloroethylene. *Ergonomics* 13(5):580–586.
- Stewart, RD; Dodd, HC; Gay, HH; et al. (1970) Experimental human exposure to trichloroethylene. *Arch Environ Health* 20(1):64–71.
- Stopps GJ, McLaughlin M (1967). Psychophysiological testing of human subjects exposed to solvent vapors. *Am Ind Hyg Assoc J.* 28(1):43-50.

*This document is a draft for review purposes only and does not constitute Agency policy.*

- Subramoniam A, Goel SK, Pandya KP, Seth PK (1989). Influence of trichloroethylene treatment on phosphoinositides in rat brain. *Toxicol Lett.* 49(1):55-60.
- Taylor DH, Lagory KE, Zaccaro DJ, Pfohl RJ, Laurie RD (1985). Effect of trichloroethylene on the exploratory and locomotor activity of rats exposed during development. *Sci Total Environ.* 47:415-40.
- Tham, R; BUNNFORS, I; Eriksson, B; et al. (1984) Vestibulo ocular disturbances in rats exposed to organic solvents. *Acta Pharmacologica Et Toxicologica* 54(1):58–63.
- Tham, R; Larsby, B; Odkvist, LM; et al. (1979) The influence of trichloroethylene and related drugs on the vestibular system. *Acta Pharmacol Toxicol (Copenh)* 44(5):336–342.
- Till C, Westall CA, Koren G, Nulman I, Rovet JF (2005). Vision abnormalities in young children exposed prenatally to organic solvents. *Neurotoxicology.* 26(4):599-613.
- Till C, Westall CA, Rovet JF, Koren G (2001). Effects of maternal occupational exposure to organic solvents on offspring visual functioning: a prospective controlled study. *Teratology.* 64(3):134-141.
- Triebig, G., S. Lehl, W. Kinzel, H. Erzigkeit, J. V. Galster and K. H. Schaller (1977a). Psycho-pathometric results of follow-up studies of persons exposed to trichloroethylene. *Zbl. Bakt. Hyg., I. Abt. Orig. B* 164, 314–327 (1977). (translated from German)
- Triebig, G., W. Bestler, P. Baumeister, H. Valentin (1983). Investigations on the neurotoxicity of chemical substances at the workplace, Part IV: Determination of the motor and sensory nerve conduction velocity in persons occupationally exposed to a mixture of organic solvents. *Int. Arch. Occup. Environ. Health*, 52, 139–150. (translated from German)
- Triebig, G; Essing, HG; Schaller, KH; et al. (1976) Biochemical and psychological examinations of trichloroethylene exposed volunteers. *Zentralbl Bakteriol [Orig B]* 163(5–6):383–416. (translated from German)
- Triebig, G; Schaller, KH; Erzigkeit, H; et al. (1977b). Biochemical investigations and psychological studies of persons chronically exposed to trichloroethylene with regard to non-exposure intervals. *Int Arch Occup Environ Health* 38(3):149–162. (translated from German)
- Triebig, G; Trautner, P; Weltle, D; Saure, E; Valentin, H. (1982). Investigations on the Neurotoxicity of processing materials III. Measurement of Motor and Sensory Nerve Conduction Velocity in Persons with Job-Related Trichloroethylene Exposure. *Int Arch Occup Environ Health* (1982) 51: 25–34 (translated from German)
- Troster, AI; Ruff, RM. (1990) Neuropsychological sequelae of exposure to the chlorinated hydrocarbon solvents trichloroethylene and trichloroethane. *Archives of Clinical Neuropsychology* 5(1):31–47.
- Truhaut, R. (1951). [Contribution to the study of toxicology of trichloroethylene; establishment of a method of determination applicable to atmosphere and biological media.] *Ann Pharm Fr.* 9:175-87.
- Umez, T; Yonemoto, J; Soma, Y; et al. (1997) Behavioral effects of trichloroethylene and tetrachloroethylene in mice. *Pharmacol Biochem Behav* 58(3):665–671.
- Valic E, Waldhör T, Konnaris C, Michitsch A, Wolf C (1997). Acquired dyschromatopsia in combined exposure to solvents and alcohol. *Int Arch Occup* 70(6):403-406.
- Vernon RJ, Ferguson RK. (1969) Effects of trichloroethylene on visual-motor performance. *Arch Environ Health* 18(6):894–900.
- Waseem, M; Ali, M; Dogra, S; et al. (2001) Toxicity of trichloroethylene following inhalation and drinking contaminated water. *J Appl Toxicol* 21(6):441–444.

*This document is a draft for review purposes only and does not constitute Agency policy.*

White RF, Feldman RG, Eviator II, Jabre JF, Niles CA (1997). Hazardous waste and neurobehavioral effects: a developmental perspective. *Environ Res.* 73(1-2):113-124.

Windemuller, FJ; Ettema, JH. (1978) Effects of combined exposure to trichloroethylene and alcohol on mental capacity. *Int Arch Occup Environ Health* 41(2):77-85.

Windham GC, Zhang L, Gunier R, Croen LA, Grether JK (2006). Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco bay area. *Environ Health Perspect.* 114(9):1438-1444.

Winneke, G. (1982) Acute behavioral effects of exposure to some organic solvents-psychophysiological aspects, *Acta Neurol Scand*, 32:117-129.

Winneke G, Kastka J, Fodor GG (1974). Psychophysiological effects of low-level exposure to trichloroethylene and toluene. Presented at the 2<sup>nd</sup> Industrial and Environmental Neurology Congress held October 22-26, 1974 in Prague. (As cited in Winneke, 1982)

Winneke G, Krämer U, Kastka J (1976), Zur Beeinflussung psychomotor Leistungen Durch Alkohol und durch verschiedene Lösungsmitteldämpfe. *Adverse Effects of Environmental Chemicals and Psychotropic Drugs*, ed. M. Horvath, 99-110, Elsevier, Amsterdam. (As cited in Winneke, 1982)

Winneke G, Fodor GG, Schlipkötter HW (1978). Carbon monoxide, trichloroethylene, and alcohol: Reliability and validity of neurobehavioral effects. *Multidisciplinary Perspectives in Event Related Brain Potential Research*, ed. D.A. Otto, 463-469, U.S. Environmental Protection Agency, Washington. (As cited in Winneke, 1982)

Wolff and Siegmund. (1978) The effect of trichloroethylene on the spontaneous locomotor activity of mice and of tetrachloroethane on the mortality of mice as a function of the time of day. *Biol. Zbl*, 97, 345-351 (As cited in Winneke, 1982).

Yamamura, K; Ikeda, I; Sadamoto, T; et al. (1983) Effects Of Trichloroethylene Exposure On Hearing. An Investigation Of Cochlear Microphonics And Action Potential Of The Guinea Pig. *European Journal of Applied Physiology and Occupational Physiology* 52:47-50.