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Subclinical abnormalities in workers with continuous low-level toluene exposure

Hsu-Tzu Shih[1,2,3], Chin-Lin Yu[2], Ming-Tsang Wu[4], Chiu-Shong Liu[3], Chon-Haw Tsai[5], Dong-Zong Hung[3], Chun-Shing Wu[6], and Hisen-Wen Kuo[2,7]

Abstract

Short-term exposure to a high concentration (TWA > 100 ppm) of toluene can cause hepatotocixity and neurotoxicity in humans. Data on the effects of exposure to low levels of toluene, however, are controversial. In addition, few studies on the effects of toluene exposure on the autonomic nervous system have been conducted. Urine samples from 34 male factory workers in Taiwan who were exposed to low levels of toluene either intermittently ($n = 13$) or continuously ($n = 21$) were taken on a Monday morning after a 2-day hiatus and at the end of the workweek on Friday evening. Urinary hippuric acid levels were measured using high-performance liquid chromatography (HPLC). A complete blood work-up was also performed for each subject. The prevalence and severity of neurotoxic symptoms were investigated by a self-reported questionnaire, a neuropsychiatric battery, and sympathetic and peripheral nerve function tests. The mean value of urinary hippuric acid corrected for creatinine (Cr) was 0.34 ± 0.18 g/g Cr on Monday morning and 0.43 ± 0.26 g/g Cr on Friday evening. The difference in the mean value of urinary hippuric acid between the two periods ($p < 0.01$) and the odds ratio of impairment of sympathetic (OR = 4.13, $p = 0.11$) and peripheral nerves (OR = 6.94, $p = 0.074$) were higher in workers continuously exposed to toluene. In addition, workers who were continuously exposed to toluene had a lower mean platelet count ($216 \pm 41 \times 10^6/\mu L$) than workers who were intermittently exposed ($252 \pm 40 \times 10^6/\mu L$), ($p = 0.018$). Furthermore, there was a positive relationship between neurological abnormalities and a self-reported neuropsychiatric measurement ($r = 0.35-0.66$, $p < 0.05$) in all workers. These data suggest that continuous exposure to low levels of toluene may be associated with sympathetic and peripheral nerve dysfunction and sub-clinical hematological damage. Further research needs to be carried out regarding how chronic exposure to low-levels of toluene affects workers.

Keywords

Toluene, hippuric acid, sympathetic skin response, questionnaire, toxicity

[1]Department of Occupational Medicine, Kuang Tien General Hospital, Taiwan
[2]Institute of Environmental Health, China Medical University, Taiwan
[3]Center for Management of Occupational Injury and Disease, China Medical University Hospital, Taiwan
[4]Graduate Institute of Occupational Safety and Health, Kaohsiung Medical University, Taiwan
[5]Department of Neurology, China Medical University Hospital, Taiwan
[6]Department of Family Medicine, Kuang Tien General Hospital, Taiwan
[7]Institute of Environmental and Occupational Health Sciences, National Yang Ming University, Taiwan

Corresponding author:
Hisen-Wen Kuo, Institute of Environmental and Occupational Health Sciences, National Yang Ming University, No.155, Sec.2, Li-Nong Street, Taipei, 112 Taiwan (ROC)
Email: occupationdr@gmail.com

Introduction

Toluene is one of the most prevalent aromatic hydrocarbons because of its extensive use as a gasoline additive. Pharmaceutical companies, rubber and adhesive industries, and technology-oriented companies use toluene as a solvent. Hippuric acid is the major metabolite from toluene breakdown by cytochrome P-450.

Short-term and long-term exposure to toluene (> 100 ppm) may damage the central nervous system, resulting in confusion, seizure, headache, vertigo, impairment of visuomotor speed, coding speed, short-term visual memory (Chouaniere et al., 2002), optic neuropathy, and poor color discrimination (Filley et al., 2004; Zavalic et al., 1998). Exposure to toluene has also been shown to induce cytochrome P450 in hepatocytes (Nakajima and Wang, 1994) and lymphocytes (Mendoza-Cantu et al., 2006), and to be associated with carcinogenesis and reproductive dysfunction (Murata et al., 1999). Thus, the National Institute for Occupational Safety and Health (NIOSH) has set the threshold limit value-time weighted average (TLV-TWA) for toluene at 50 ppm. It has been shown, however, that even sub-chronic toluene exposure can result in cerebellar atrophy (Filley et al., 2004) and deterioration of memory and attention (Lee et al., 2003; Kang et al, 2005). Murine models have revealed that exposure to low levels (40−80 ppm) of toluene results in memory loss and impaired visuospatial learning (Von et al., 1993), damage to neuroglial cells (Burry et al., 2003), and significant and gender-dependent alterations in the autonomic nervous system (ANS) related to the rate of catecholamine and 5-hydroxytryptamine biosynthesis in brainstem catecholaminergic cell groups and in the hypothalamus (Soulage et al., 2004; Berenguer et al., 2003a). The prevalence of toluene-related neurotoxicity among workers exposed to toluene is still unclear because of controversial data on neurotoxicity arising from low-dose chronic toluene exposure (<50 ppm; Eller et al., 1999;Gericke et al., 2001; Juntunen et al., 1985; Murata et al., 1994; Seeber et al., 2004; Zupanic et al., 2002). Thus, there is a need to know whether workers with low-level toluene exposure have symptoms related to neurotoxicity and whether the presence of toxicity depends on the mode of exposure. In the present study, the prevalence and severity of neurotoxic symptoms among workers exposed to low levels of toluene were investigated by a self-reported questionnaire, a neuropsychiatric battery, and sympathetic and peripheral nerve function tests.

Materials and methods

Subjects

During the period October 2006 to December 2006, we recruited 34 male workers from two departments (A and B) of a factory established in 2003 in Taiwan. Thirteen workers in department A were exposed to toluene intermittently while mixing raw materials (2−3 hours per day) and twenty-one workers in department B were continuously exposed each day during the entire workweek (Monday to Friday). All workers worked 12 hours per day, 5 days per week, and wore masks when exposed to toluene. None of the workers had any history of alcohol or drug addiction, neuropsychiatric disorders, thyroid disease, or renal or liver insufficiency. All workers signed a consent form.

Biological monitoring

Each subject provided two urine specimens, one on Monday morning before starting their shift after a 2-day hiatus (baseline) and the other one at the end of their shift on Friday evening. None of the subjects were allowed to consume sedatives, salicylic acid, alcohol, or black tea for at least 10 hours or to smoke for at least 2 hours prior to collection of the urine specimens. Hippuric acid levels in urine were measured by high-performance liquid chromatography (HPLC; Ogata and Taguchi, 1986). Urinary creatinine levels were measured using standard laboratory procedures. The ratio of urinary hippuric acid to creatinine was used to represent the level of toluene exposure among the study subjects in the workplace. We used the current American Conference of Governmental Industrial Hygienists (ACGIH) biological exposure index (BEI) of 1.6g/g creatinine as a cutoff value for post-exposure urine.

Examination of health effects

All subjects received a health examination at a.m. 10:00−12:00 on the third workday (14−16 hours after leaving the factory) and all were required to avoid sleep deprivation or unusual fatigue prior to the examination.

Blood test

Blood samples were taken in the morning on the day of the examination and analyzed for complete blood count, glucose level, and liver, renal, and thyroid function. All data were assessed at a medical center.

Nerve functions

Nerve functions were assessed by a trained technician using standard methods. Sympathetic skin response (SSR) is a measure of sudomotor activity and is based on changes in skin conductance levels in response to various internal or external stimuli. SSR has been used to assess sympathetic dysfunction in diabetic patients, in patients with peripheral neuropathies (Kiylioglu et al., 2005), and in patients with diseases affecting the central nervous system (Sharma et al., 1999). During the SSR procedure in this study, the ambient temperature was maintained at 24−25°C and the active electrodes were placed on the subjects' bilateral palms and soles. Then, 4−7 electric stimulations with a 60-mA intensity were delivered to the right median nerve at 20−40 s intervals. The best of three responses with greater reproducibility was selected and the latency of the evoked potential was examined in the subjects' four limbs. A prolonged latency was indicative of poor sympathetic function. A latency exceeding 1800 ms in the upper extremity or more than 4000 ms in the lower extremity was considered abnormal. Sensory nerve conduction velocity (SNCV) of the bilateral median nerve was also measured at the wrist to evaluate peripheral nerve function. Low conduction velocity was indicative of poor peripheral nerve function. A conduction velocity lower than 48 m/s was considered abnormal.

Neuropsychological tasks

Two tests from the computerized test battery "Neuroscan 2003 Stim^2-stimulus presentation system" were chosen. The cued attention test was used to explore the orientation of visual-spatial attention. The finger-tapping test was used to assess fine upper-limb motor performance and manual dominance. All subjects were seated in a comfortable position in front of the keyboard while performing the tests (Jobbagy et al., 2005).

Cued attention test. A two-target detection paradigm with a fixed point was shown in the center of the screen. After a short intertribal interval, a highlighted visual cue in the shape of a box was presented peripherally to predict the location of a target stimulus. After a short interstimulus interval, a target stimulus appeared in one of two screen locations. The workers needed to correctly identify the location of the target by pressing a response button. The cueing conditions were classified into three groups (valid, invalid, and no cue). For valid trials, the target appeared in the screen location indicated by the cue. For invalid trials, the target appeared in the region opposite the cue. For no cue trials, the cue was absent. The probability of each trial type was under program control and 240 trials were done. The average reaction time and error rate values were recorded.

Finger tapping test. The test administrator compiled 2 separate scores from the complete test procedure. One set of scores represented the right hand (10 seconds) and the other set represented the left hand (10 seconds). Workers were asked to tap the keyboard with their index finger as quickly as possible. The right hand was used first followed by the left hand. Each test was performed in triplicate. The average number of finger tappings in the dominant and non-dominant hand was recorded.

Questionnaire on neurotoxic symptoms and health

A comprehensive self-reported questionnaire included questions about the worker's history, consumption of alcohol, smoking habits, and medical history. Each worker completed the Chinese Version of the Modified European Questionnaire (EUROQUEST) in the morning on the day of the neurological examination. The original EUROQUEST was developed in 1992 to explore the most commonly reported neurotoxic symptoms associated with long-term occupational solvent exposure (Carter et al., 2002; Karlson et al., 2000) and was modified in 2004 (Kaukiainen et al., 2004). It is a sensitive screening instrument for identifying toxic encephalopathy and consists of six domains. The scores range from 34 to 170. The domains include neurological symptoms (6 items), psychosomatic symptoms (9 items), mood lability (7 items), memory and concentration difficulties (6 items), tiredness (2 items), and sleep disturbance (4 items). Subjects were asked to rate the frequency in which they experienced the symptoms listed in the six domains during the previous six months. The items were measured using a 5-point Likert-type scale. The answers were coded as "never", "once per month", "once per week", "more than twice per week," and "occurs every day."

Statistical analysis

The paired-sample T test was used to compare mean values of hippuric acid at baseline and at the end of

Table 1. Comparison of subject characteristics and levels of urine hippuric acid at baseline and at the end of the workweek between departments A and B

	Dep A ($N = 13$)	Dep B ($N = 21$)	p
Age (mean ± SD)	25.15 ± 2.15	27.10 ± 5.59	0.24
Work duration (mean ± SD)	10.88 ± 10.84	9.50 ± 10.17	0.73
Cigarette smoking (n/%)	7 (53.85%)	14 (66.67%)	0.70[b]
Alcohol drinking (n/%)	2 (15.38%)	4 (19.05%)	1.00[b]
Urine hippuric acid before-work (mean ± SD)	0.30 ± 0.17	0.36 ± 0.19	0.42
After work (mean ± SD)	0.37 ± 0.22	0.46 ± 0.27	0.41
p	0.54[a]	0.006[a]	

Comparisons by independent-sample T test, except
[a]p-values represent the statistical results of paired-sample T test.
[b]p-values represent the statistical results of Chi-square and Fisher's exact tests.

the workweek. The independent-sample T test was used to compare the mean value of urine hippuric acid, latency of SSR, conduction velocity of SNCV, reaction time during the cued attention test, and hematologic parameters between workers in department A and those in department B. The chi-square and Fisher's exact tests were used to compare the percentage of workers with current habits of alcohol consumption and cigarette smoking in department A with those in department B. Odds ratios (OR) were calculated to evaluate the risk of neurological function impairment in departments A and B. The Spearman's correlation coefficient was used to analyze the correlation between work duration, self-reported neurotoxic symptom scores, and results of the neurological examination. A two-tailed p value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed on a personal computer with the statistical package SPSS for Windows (Version 13.0, SPSS, Chicago, Illinois, USA).

Results

General characteristics of the study population

The mean age of the 34 workers was 26.4 years, and the mean work duration was 10 months. The duration of work was short with high variation (1−37 months). There was no difference in socioeconomic and educational status between workers of two departments. The mean value of urinary hippuric acid was higher in specimens at the end of the workweek (0.34 ± 0.18 g/g Cr Vs 0.43 ± 0.26 g/g Cr). All urinary hippuric acid levels were below the ACGIH BEI of 1.6 g/g Cr in post-exposure urine. The levels of toluene exposure in the workplace did not exceed the TLV-TWA of 100 ppm set by the NIOSH in Taiwan. There were no significant differences in percentage of workers who drank alcohol more than once per week or smoked cigarettes between workers in department A and those in department B (Table 1)

Biological monitoring—hippuric acid level in urine

There was no significant difference in mean level of urinary hippuric acid on Monday morning and Friday evening between the two departments; however, the difference in mean urinary hippuric acid level on Monday morning and Friday evening was significantly higher in department B than in department A (Table 1).

Evaluation of health effects

Blood test. The mean platelet count was significantly lower in workers in department B (216 ± 41 × $10^6/\mu L$) than in workers in department A (252 ± 40 × $10^6/\mu L$) ($p = 0.018$; Table 2). The results of the other blood tests did not differ significantly between the two departments.

Nerve function test. In the upper extremities, the latency of evoked potential of sympathetic skin response was shorter than 1800 ms in all subjects, and there was no significant difference in the mean latency between the two departments. In the lower extremities of the 21 workers in department B, eight had a latency of more than 4000 ms (38.1%), whereas only 2 of the 13 workers in department A had such a manifestation (15.4%; Table 2). The odds of having an abnormal SSR was more than four times greater in department B than in department A (OR, 4.13; 95% CI = 0.73−23.43, $p = 0.11$).

Table 2. Comparison of blood tests, sympathetic, peripheral, neurobehavior function tests, and scores of questionnaires between workers in the two exposure groups

	Dep A (N = 13) Mean ± SD	Dep B (N = 21) Mean ± SD	p
Platelet count ($10^6/\mu L$) (mean ± SD)	252 ± 40	216 ± 41	0.018
WBC ($10^3/\mu L$; mean ± SD)	5.77 ± 1.05	5.88 ± 1.46	0.80
RBC ($10^6/\mu L$; mean ± SD)	5.10 ± 0.48	5.17 ± 0.55	0.73
SSR, upper limb			
Onset latency, right (ms; mean ± SD)	1352 ± 159	1384 ± 189	0.61
Onset latency, left (ms; mean ± SD)	1366 ± 159	1430 ± 277	0.45
SSR, lower limb			
Abnormal response (n/%)	2 (15.4%)	8 (38.1%)	0.11[a]
SNCV, bilateral median nerve			
Right (m/s; mean ± SD)	56.31 ± 5.44	53.86 ± 5.03	0.19
Left (m/s; mean ± SD)	58.00 ± 7.00	54.86 ± 5.47	0.15
Abnormal response (n/%)	0 (0%)	4 (19.0%)	0.074 [a]
Neuropsychological tasks			
Cued attention test			
No-cue (ms; mean ± SD)	0.37 ± 0.075	0.35 ± 0.045	0.51
Valid cue (ms; mean ± SD)	0.32 ± 0.030	0.33 ± 0.036	0.63
Invalid cue (ms; mean ± SD)	0.36 ± 0.052	0.35 ± 0.033	0.59
Finger tapping			
Dominant hand (times/sec)	7.47 ± 0.80	7.60 ± 1.24	0.74
Non-dominant hand(times/sec)	6.19 ± 1.09	6.45 ± 1.35	0.56
Score of Questionnaire (mean ± SD)			
Neurological symptoms	8.92 ± 3.01	9.14 ± 3.79	0.86
Psychosomatic symptoms	15.69 ± 4.59	14.05 ± 4.06	0.28
Mood lability	13.85 ± 3.89	13.10 ± 3.81	0.58
Memory and concentration difficulties	11.15 ± 3.83	11.43 ± 4.89	0.86
Tiredness	4.54 ± 1.71	4.81 ± 2.06	0.70

Comparisons by independent-sample T test, except
[a]p-values represent the statistical results of Logistic regression with odds ratio.
SSR: sympathetic skin response; SNCV: sensory nerve conduction velocity.

The conduction velocity of the sensory nerve action potential (SNCV) in the bilateral median nerve was recorded. Although there was no significant difference between the departments, there was a relative reduction in the conduction velocity among subjects in department B. Of the 21 workers in department B, 4 had a conduction velocity of less than 48 m/s (19%), whereas none of the subjects in department A had such a manifestation (0%; Table 2). There was no difference in the mean SNCV between individuals with a normal SSR and those with an abnormal SSR ($p = 0.26$).

Neuropsychological tasks. The error rate of the cued attention test was less than 1% and the reaction time was within the normal range in all workers. There was no significant difference in the mean reaction time, or the mean error rate of the cued attention test, and no significant difference between the mean frequency of finger tapping between the workers in the two departments (Table 2).

Scores of self-reported modified EUROQUEST

There were no significant differences in the scores of the six symptom scales between the two exposure groups (Table 2). Significant correlations were found between neurological symptoms and the other five domains: psychosomatic symptoms ($r = 0.45$, $p = 0.008$), mood liability ($r = 0.53$, $p = 0.001$), memory and concentration difficulties ($r = 0.56$, $p = 0.001$), tiredness ($r = 0.40$, $p = 0.019$), and sleep disturbance ($r = 0.39$, $p = 0.025$). There was also a significantly positive relationship between the onset latency of SSR in department B and the scores of the six domains of the questionnaire ($r = 0.35$ to 0.65). There was also a negative correlation between sensory nerve conduction velocity and the neurological symptom and sleep disturbance domain scores (Table 3).

Table 3. Correlation between questionnaire data and neurological examinations

	Sympathetic skin response, sensory nerve conduction velocity			
EUROQUEST Domain Score	SSR RH (r)	SSR LH (r)	SNCV RH (r)	SNCV LH (r)
Dep A ($N = 13$)				
Neurological s/s	−0.09	−0.24	0.27	0.24
Psychiatric s/s	0.49	0.19	0.40	0.48
Mood disturbance	0.27	0.24	0.13	0.12
Attention/Memory	−0.13	−0.06	0.28	0.13
Tiredness	0.14	0.08	0.12	0.07
Sleep disturbance	−0.01	0.17	0.25	0.02
Dep B ($N = 21$)				
Neurological s/s	0.65***	0.62***	−0.52*	−0.48*
Psychiatric s/s	0.48*	0.51*	−0.26	−0.18
Mood disturbance	0.61**	0.66***	−0.32	−0.12
Attention/memory	0.41	0.45*	−0.27	−0.07
Tiredness	0.47*	*0.35*	−0.21	−0.09
Sleep disturbance	0.45*	0.59**	−0.56*	*-0.31*

Analysed by Spearman's correlation.
*$p < 0.05$, **$P < 0.01$, ***$P < 0.001$, oblique type word: $p < 0.1$.
SSR: sympathetic skin response; SNCV: sensory nerve conduction velocity.
RH: right hand; LH: left hand; r: Correlation coefficient.

Discussion

It has been reported that the hippuric acid level in urine is 0.6 g/g creatinine in subjects exposed to a toluene concentration of 40 ppm (Ogata and Taguchi, 1986). In the present study, the mean level of hippuric acid in post-shift urine specimens, when corrected for creatinine, was 0.43 ± 0.26 g/g Cr. This indicates that the mean air concentration of toluene in the factory sampled in this study was less than 40 ppm and, therefore, within the limit set in Taiwan.

Urinary hippuic acid is the most common biomarker for monitoring toluene exposure. It is valid when the toluene exposure level is high (>50 ppm); however, the marker correlates poorly with exposure when the level of toluene exposure is less than 10 ppm (Ikeda et al., 2008; Ukai et al., 2007). In addition, alcohol and cigarette smoking interact with toluene metabolism and have both stimulatory (chronic consumption) and inhibitory (acute consumption) effects on the metabolism of toluene (Inoue et al., 1993). In the present study, all subjects were instructed to refrain from using any confounding substances prior to urine specimen collection in an attempt to clarify the real effect of occupational toluene exposure. Furthermore, the ratio of workers with current habits of alcohol consumption and cigarette smoking did not differ between the two departments. Therefore, the significant difference in urinary hippuric acid levels at baseline and at the end of the workweek in workers in department B might indicate a higher level of occupational exposure to toluene in that department.

Few studies on the hematological effects of low-level toluene exposure have been conducted. It has been reported that low-level toluene exposure can promote transient platelet agglutination, which can result in pseudo-thrombocytopenia (Aakhus et al., 1991). Therefore, in the present study, the reduction in platelet count in workers with long-term continuous toluene exposure could be due to transient hyper-agglutination, disturbance of platelet synthesis, or due to increasing platelet damage. Our findings might support the notion that platelet count may serve as an effective means by which to monitor the effects of low-level toluene exposure.

Workers with chronic exposure to toluene show a significant reduction in the electrocardiographic R-R interval and in the SNCV in the median palmar nerve (Murata et al., 1993). Chronic exposure to low-dose mixed-solvents, such as *n*-hexane, xylene, and toluene, affects cardiac parasympathetic activity and reduces the peripheral nerve conduction velocity (Murata et al., 1994). In the present study, higher odds ratio of having an abnormal SNCV in department B compared with department A might indicate that subjects who are exposed to continuous low levels of toluene are at marginally higher risk of developing peripheral sensory nerve function impairment.

To the best of our knowledge, no studies on the effects of toluene exposure on the sympathetic nervous system have been conducted. SSR has the potential to detect subclinical ANS dysfunction, and studies reveal that the most common abnormality is unilateral prolongation of lower extremity latency (Nazliel et al., 2007). In the present study, SSR of the upper extremities was intact in all subjects but could not be elicited in lower extremities in 29.4% of our subjects, especially in those with continuous exposure (38.1%). This high ratio of sympathetic functional impairment is unusual in young, healthy men. The SSR includes 3 phases, somatosensory myelinated afferents, central coupling, and efferent controlled output. Since there was no difference in the mean SNCV between subjects with a normal SSR and those with an abnormal SSR, the abnormalities observed in the present study are most likely related to dysfunction of the central coupling process or efferent pathways (hypothalamus, brainstem catecholamine and 5-hydroxytryptamine biosynthesis cell groups, limbic system and spinal cord) (Berenguer et al., 2003b; Soulage et al., 2004; Sharma et al., 1999).

Exposure to mixed organic solvents has been shown to lead to poorer memory and fine motor performance in children (Saddik et al., 2005), whereas low-level toluene exposure in adults (<50 ppm) who work in the printing industry has been reported to be associated only with decrements of memory test performance. In that study, there was no significant impairment in perceptual motor or attention function (Seeber et al., 2005). We found similar results. Thus, low-level toluene exposure apparently does not affect the orientation of visual-spatial attention or fine upper-limb motor performance.

In workers with continuous toluene exposure, there is a significant negative correlation between SNCV and the neurological symptom-scale and sleep disturbance-scale scores. One possible explanation for this finding is that patients with peripheral neuropathy may suffer from fullness and numbness in the hands, which can result in sleep disturbance (Afshar et al., 2007). We also found a positive correlation between the scores of all domains and the onset latency of SSR. This finding supports that reported by Newton JL, who reported that autonomic dysregulation is associated with poor sleep quality, fatigue, restlessness, and mood liability (Newton et al., 2007). Chronic exposure to toluene might, therefore, result in neurological damage and subjective discomfort.

The limitations to this study include its cross-sectional study design and small sample size, making it difficult to clearly establish a relationship between psychomotor function and exposure to low levels of toluene. In addition, the presence of weak effects in sympathetic and peripheral nerve function as well as platelet counts indicates the need for further investigation using a longitudinal approach. Furthermore, the existence of a good correlation between the questionnaire and neurological examinations might support the notion that biological monitoring and a self-reported questionnaire might be valid methods to quickly screen for early neurological damage in workers with continuous exposure to low levels of toluene.

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References

Aakhus AM, Smith-Kielland A, Ripel A, and Solum NO (1991) Effects of toluene on platelet membrane glycoprotein Ib and actin-binding protein. *Biochemical Pharmacology* 42(4): 805-811. 1991.

Afshar A, Yekta Z, and Mirzatoluei F (2007) Clinical course of the non-operated hand in patients with bilateral idiopathic carpal tunnel syndrome. *Journal of Hand Surgery–American* 32(8): 1166-1170.

Berenguer P, Soulage C, Perrin D, Pequignot JM, and Abraini JH (2003a) Behavioral and neurochemical effects induced by subchronic exposure to 40 ppm toluene in rats. *Pharmacology, Biochemistry, and Behavior* 74: 997-1003.

Berenguer P, Soulage C, Perrin D, Pequignot JM, and Abraini JH (2003b) Behavioral and neurochemical effects induced by subchronic exposure to 40 ppm toluene in rats. *Pharmacology, Biochemistry, and Behavior* 74: 997-1003.

Burry M, Guizzetti M, Oberdoerster J, and Costa LG (2003) Developmental neurotoxicity of toluene: in vivo and in vitro effects on astroglial cells. *Developmental Neuroscience* 25: 14-19.

Carter N, Iregren A, Soderman E, Olson B A, Karlson B, Lindelof B, et al (2002) EUROQUEST–a questionnaire for solvent related symptoms: factor structure, item analysis and predictive validity. *Neurotoxicology* 23: 711-717.

Chouaniere D, Wild P, Fontana JM, Hery M, Fournier M, Baudin V, et al. (2002) Neurobehavioral disturbances

arising from occupational toluene exposure. *American Journal of Industrial Medicine* 41: 77-88.

Eller N, Netterstrom B, and Laursen P (1999) Risk of chronic effects on the central nervous system at low toluene exposure. *Occupational Medicine (London)* 49: 389-395.

Filley CM, Halliday W, and Kleinschmidt-DeMasters BK (2004) The effects of toluene on the central nervous system. *Journal of Neuropathology and Experimental Neurology* 63: 1-12.

Gericke C, Hanke B, Beckmann G, Baltes MM, Kuhl KP, and Neubert D (2001) Multicenter field trial on possible health effects of toluene. III. Evaluation of effects after long-term exposure. *Toxicology* 168: 185-209.

Ikeda M, Ukai H, Kawai T, Inoue O, Maejima Y, Fukui Y, et al (2008) Changes in correlation coefficients of exposure markers as a function of intensity of occupational exposure to toluene. *Toxicology Letters* 179: 148-154.

Inoue O, Seiji K, Watanabe T, Nakatsuka H, Jin C, Liu SJ, and Ikeda M (1993) Effects of smoking and drinking on excretion of hippuric acid among toluene-exposed workers. *International Archives of Occupational and Environmental Health* 64: 425-430.

Jobbagy A, Harcos P, Karoly R, and Fazekas G (2005) Analysis of finger-tapping movement. *Journal of Neuroscience Methods* 141(1): 29-39.

Juntunen J, Matikainen E, Antti-Poika M, Suoranta H, and Valle M (1985) Nervous system effects of long-term occupational exposure to toluene. *Acta Neurologica Scandinavica* 72: 512-517.

Kang SK, Rohlman DS, Lee MY, Lee HS, Chung SY, and Anger WK (2005) Neurobehavioral performance in workers exposed to toluene. *Environmental Toxicology and Pharmacology* 19: 645-650.

Karlson B, Osterberg K, and Orbaek P (2000) Euroquest: the validity of a new symptom questionnaire. *Neurotoxicology* 21: 783-789.

Kaukiainen A, Riala R, Martikainen R, Akila R, Reijula K, and Sainio M (2004) Solvent-related health effects among construction painters with decreasing exposure. *American Journal of Industrial Medicine* 46: 627-636.

Kiylioglu N, Akyol A, Guney E, Bicerol B, Ozkul A, and Erturk A (2005) Sympathetic skin response in idiopathic and diabetic carpal tunnel syndrome. *Clinical Neurology and Neurosurgery* 108: 1-7.

Lee YL, Pai MC, Chen JH, and Guo YL (2003) Central neurological abnormalities and multiple chemical sensitivity caused by chronic toluene exposure. *Occupational Medicine (London)* 53: 479-482.

Mendoza-Cantu A, Castorena-Torres F, Bermudez de LM, Cisneros B, Lopez-Carrillo L, et al. (2006) Occupational toluene exposure induces cytochrome P450 2E1 mRNA expression in peripheral lymphocytes. *Environmental Health Perspectives* 114: 494-499.

Murata K, Araki S, Yokoyama K, Tanigawa T, Yamashita K, Okajima F, et al. (1993) Cardiac autonomic dysfunction in rotogravure printers exposed to toluene in relation to peripheral nerve conduction. *Industrial Health* 31: 79-90.

Murata K, Araki S, Yokoyama K, Yamashita K, Okajima F, and Nakaaki K (1994) Changes in autonomic function as determined by ECG R-R interval variability in sandal, shoe and leather workers exposed to n-hexane, xylene and toluene. *Neurotoxicology* 15: 867-875.

Murata M, Tsujikawa M, and Kawanishi S (1999) Oxidative DNA damage by minor metabolites of toluene may lead to carcinogenesis and reproductive dysfunction. *Biochemical and Biophysical Research Communications* 261: 478-483.

Nakajima T and Wang RS (1994) Induction of cytochrome P450 by toluene. International Journal of Biochemistry 26: 1333-1340.

Nazliel B, Arikan Z, Irkec C, and Karakilic H (2007) SSR abnormalities in chronic alcoholics. *Addictive Behaviors* 32(6): 1290-1294.

Newton JL, Okonkwo O, Sutcliffe K, Seth A, Shin J, and Jones DE (2007) Symptoms of autonomic dysfunction in chronic fatigue syndrome. *QJM* 100(8): 519-526. 2007.

Ogata M and Taguchi T (1986) Quantitative analysis of urinary glycine conjugates by high performance liquid chromatography: excretion of hippuic acid and methylhippuric acid in the urine of subjects exposed to vapours of toluene and xylenes. *International Archives of Occupational & Environmental Health* 58: 121-129.

Saddik B, Williamson A, Nuwayhid I, and Black D (2005) The effect of solvent exposure on memory and motor dexterity in working children. *Public health reports* 120: 657-663.

Seeber A, Demes P, Kiesswetter E, Schaper M, van TC, and Zupanic M (2005) Changes of neurobehavioral and sensory functions due to toluene exposure below 50 ppm? *Environmental Toxicology and Pharmacology* 19: 635-643.

Seeber A, Schaper M, Zupanic M, Blaszkewicz M, Demes P, Kiesswetter E, et al. (2004) Toluene exposure below 50 ppm and cognitive function: a follow-up study with four repeated measurements in rotogravure printing plants. *International Archives of Occupational and Environmental Health* 77: 1-9.

Sharma KR, Romano JG, Ayyar DR, Rotta FT, Facca A, and Sanchez-Ramos J (1999) Sympathetic skin response and heart rate variability in patients with Huntington Disease. Archives of Neurology 56: 1248-1252.

Soulage C, Perrin D, Berengure P, and Pequignot JM (2004) Sub-chronic exposure to toluene at 40 ppm alters the monoamine biosynthesis rate in discrete brain areas. *Neurotoxicology* 196: 21-30.

Ukai H, Kawai T, Inoue O, Maejima Y, Fukui Y, Ohashi F, et al. (2007) Comparative evaluation of biomarkers of occupational exposure to toluene. *International Archives of Occupational and Environmental Health* 81: 81-93.

Von EG, Ogren SO, Li XM, Fuxe K, and Gustafsson JA (1993) Persistent effects of subchronic toluene exposure on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D2 agonist binding in the rat. *Toxicology* 77: 223-232.

Zavalic M, Mandic Z, Turk R, Bogadi-Sare A, Plavec D, and Skender LJ (1998) Qualitative color vision impairment in toluene-exposed workers. *International Archives of Occupational and Environmental Health* 71: 194-200.

Zupanic M, Demes P, and Seeber A (2002) Psychomotor performance and subjective symptoms at low level toluene exposure. *Occupational and Environmental medicine* 59: 263-268.