

# Cognitive functions changes among Egyptian sewage network workers

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## Abstract

The current work aimed at investigating the cognitive functions impairment among workers of sewer networks due to exposure to hydrogen sulfide (H<sub>2</sub>S) and the relation of this impairment, if any, to the level of H<sub>2</sub>S exposure biomarker 'urinary thiosulfate.' Besides, the validity of using Mini Mental State Examination (MMSE) as screening test for cognitive impairment among the exposed workers was tested. The work was conducted among 33 sewage network maintenance male workers and a matched unexposed control group ( $n = 30$ ). The participants were subjected to clinical neurological history, estimation of urinary thiosulfate, and assessment of cognitive dysfunction by using neurophysiological (simple reaction time, P300 test) and neuropsychological tests (Wechsler Memory Scale) and frontal executive functions tests. Clinical neurological history revealed significantly higher neurological symptoms (headache, memory defects, lack of concentration) among exposed workers compared to their controls ( $p < 0.05$ ). Exposed workers had significantly prolonged simple reaction time and delayed P300 latency and showed poor performance of most of neuropsychological tests. Marked elevation of urinary thiosulfate was observed among the exposed workers ( $p < 0.001$ ) but this elevation was not correlated with the duration of exposure or any of the other measured parameters. Exposed workers had significantly lower mean value of MMSE scoring than that of the controls ( $p < 0.001$ ). In conclusion, exposure to H<sub>2</sub>S among sewer network workers is associated with cognitive impairment, which can be screened by applying MMSE as a simple rapid test for H<sub>2</sub>S occupationally exposed workers.

## Keywords

hydrogen sulfide, sewer networks, thiosulfate, MMSE, P300, neuropsychological test, frontal executive functions

## Introduction

Hydrogen sulfide (H<sub>2</sub>S) is a highly toxic, colorless gas smelling of rotten eggs. It is used in several industries and is a product of many industrial processes such as oil refining, mining, and rayon manufacture (Fenga et al., 2002). Because H<sub>2</sub>S is produced through decomposition of organic matter by bacteria, it represents one of the most serious chemical hazards that face sewer workers, particularly in confined places such as sewer pipes (Saad et al., 2003).

Exposure to H<sub>2</sub>S occurs primarily by inhalation but can also occur by ingestion (contaminated food) and through skin (water and air). Once taken into the body, it is rapidly distributed to various organs, including the central nervous system, lungs, liver, muscle. The major metabolic pathway for H<sub>2</sub>S in the body is oxidation of sulfide to sulfate with the sulfate being excreted in the urine in the form of thiosulfate

(EPA, 2003). H<sub>2</sub>S and hydrogen cyanide are known to compromise mitochondrial respiration through inhibition of cytochrome oxidase and this is generally considered to be their primary mechanism of toxicity. This prevents the utilization of oxygen with an uncoupling of oxidative phosphorylation. Thus, the electron transport chain is disrupted by preventing oxygen from acting as the final electron acceptor and causing blockage of oxidative metabolism, leading to

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anaerobic metabolism, decreased ATP production with curtailed cellular energy generation, and the generation of lactic acid (Thompson et al., 2003). Nervous and cardiac tissues, which have the highest oxygen demand, are especially sensitive to the disruption of oxidative metabolism. This explains the occurrence of sudden death in case of exposure to high concentration (Yalamanchili and Smith, 2008).

Many studies have documented the neurological effects following exposures to non-fatal high concentrations of H<sub>2</sub>S. These effects may be permanent or persistent and include visual and memory impairment, reduced motor function, slight tremor, ataxia, psychosis, abnormal learning, retention, and motor function, and slight cerebral atrophy (ATSDR, 2004). Kilburn (2004) reported that sensitive testing showed impaired brain functions among acute H<sub>2</sub>S poisoning survivors who had been unconscious and looked all right after that.

Contrarily, long-term effects of occupational exposure to low levels of the gas have not been studied enough. However, (Kilburn, 2001) reported that long-term exposure to low concentrations of H<sub>2</sub>S is associated with neuropsychiatric and cognitive dysfunction manifestations, including: fatigue, loss of appetite, irritability, impaired memory, altered mood states, headache, and dizziness. However, it is not known if these effects are the result of chronic exposure or due to reoccurring acute exposures.

Therefore, this study aims to investigate the cognitive function changes among sewer network workers (as an example to the chronic occupational exposure to H<sub>2</sub>S) who do not have history of unconsciousness. In addition, the study investigates the validity of using thiosulfate (H<sub>2</sub>S exposure biomarker) and Mini Mental State Examination (MMSE) (non-specific cognitive function test) as screening tests for detection of early cognitive function changes among H<sub>2</sub>S-exposed personnel.

## Participants

This study was carried out among all the workers involved in maintenance of the sewage network of the districts El Manial, Misr el Kadema, and Kasr El-Aini hospitals ( $n = 42$ ). When the workers were asked to join our research, four refused and three were excluded according to our selection criteria, so the study started with 35 male workers. The selection criteria were that the workers must have been regularly working in sewer networks for at least the preceding 5 years and had no

history of unconsciousness in relation to work. After taking medical history and applying clinical examination, additional 2 workers were excluded as they had advanced hepatic diseases.

The workers were responsible for inspection of lines in order to detect any perturbation of the wastewater flow and possible defaults (for example, cracks, leaks) of the duct, the curing of the wastewater lines and the extraction of the sediments. Their frequency of exposure was about 3-4 times/week. None of the participants used to wear protective masks during work. All of the participants were literate.

The controls were recruited among personnel working in different administrative jobs that do not carry the risk of exposure to H<sub>2</sub>S gas. The controls were chosen as to be matched with exposed workers regarding age, educational level and socio-economic status. The nature of the study was fully explained to each participant and accordingly oral consent was obtained.

## Methods

Every participant completed a questionnaire sheet including personal, occupational history. Past history included chronic neurological diseases such as cerebrovascular stroke, Parkinsonism, intracranial neoplasms, major medical diseases such as renal, hepatic, metabolic disorders, known cardiac disease, other endocrinal disorders, and associated collagen vascular disorders. Symptoms suggestive of possible cognitive impairment, namely memory defects, lack of concentration, and headache were included in the questionnaire. Each participant was subjected to both general and neurological examination.

### *Estimation of urinary thiosulfate*

Thiosulfate is determined in urine after removal of interfering compounds including endogenous thiocyanate by ion exchange. First, thiosulfate is converted to thiocyanate in the presence of cyanide and cupric ions, then, the formed thiocyanate is concentrated and diluted with acid solution of ferric ion. Ferric thiocyanate using calorimetry at 500 nm wave length (Sorbo and Ohman, 1978).

### *Neurophysiological tests*

*Auditory event-related potentials (P300 component).* Event-related potentials (ERP) represent the neural activity generated during cognitive process. P300 recorded in a sound-attenuated room (Oddball paradigm),

4-channel computer average (Schwarzer myos 2 and myos 4) was used to record the responses. The hearing threshold for each subject was determined. A total of 200 auditory stimuli (bursts) were presented to the ears through earphones with intensity of 60 db above the hearing threshold. Seventy percentage of tones were 1000 Hz in frequency (background tones) whereas the remaining 30% were 2000 Hz (target tones)

The subject was instructed to press on a button connected to the apparatus as quickly as possible whenever hearing the infrequent tones. These tones were presented randomly intermixed at a rate of 0.5/sec. The tones were applied so as no two target tones came in succession.

The wave P300 was defined as the most positive point of the average waveform to the target tones after 250 msec and before 600 msec. To verify producibility of the response, the procedure was repeated at least once. The responses were displayed on a screen and could be printed out. For each subject, P300 latency, P300 amplitude, and Reaction Time were obtained to detect any significant abnormality. Latency range of P300 can be found is 250–500 msec for most adult subjects between 20 and 70 years (Niedermeyer and Lopes Da Silva, 1999).

### Neuropsychological assessment

*Wechsler Memory Scale-revised (WMS-R; Wechsler, 1981)*. The Wechsler Memory Scale was for many years the standard tool for the assessment of suspected memory disorders. It consists of 13 subtests. However, in this work, we selected the subtests that assess the visual memory and the logical memory:

1. Visual memory subtests:
  - (a) Figural memory: The maximum score is 10.
  - (b) Visual Paired Associates I: Maximum score is 18.
  - (c) Visual Reproduction I: Maximum score is 4.

The previous three tests assess the non-verbal working memory.

- (d) Digit span:
 

Digit forward (Normal:  $6 \pm 1$ , Bordline or impaired: 4, Defective: 3).

Digit backward (Normal:  $5 \pm 1$ , Bordline or impaired: 3, Defective: 2).

This test measures attention process and short-term auditory verbal (working) memory

2. Logical memory subtest:

The total scoring of 50 and patient who scores <25 is considered to have learning and working memory difficulties. This test examines the verbal memory

- B. Frontal executive functions: they were tested by
  1. Verbal fluency (Benton, 1968)

It assesses the patient's ability to generate responses by having them produce as many words as possible in 1 minute related to certain category, beginning with the names of foods, then names of animals. It tests for semantic memory. Persons who scores <15 words on the average scores of foods and animals names were showed to have impairment on this task. Scoring of <10 words indicates severe memory defects.

2. Trail making A test (Reitan, 1958)

Score 1 or 0. It tests for attention

*Mini-Mental State Examination (MMSE; Folstein et al., 1975)*. The MMSE is a fully structured scale that consists of 30 points grouped into seven categories: orientation to place (state, county, town, hospital, and floor), orientation to time (year, season, month, day, and date), registration (immediately repeating three words), attention and concentration (serially subtracting 7, beginning with 100, or, alternatively, spelling the word world backward), recall (recalling the previously repeated three words), language (naming two items, repeating a phrase, reading aloud and understanding a sentence, writing a sentence, and following a three-step command), and visual construction (copying a design). The tests take 10 min. The total score was 30 for educated persons and score of 24 is taken as a cutoff point to diagnose cognitive impairment.

The aim of the MMSE in this study was to assess the validity of using the test for screening of cognitive dysfunction resulting from occupational exposure to H<sub>2</sub>S.

### Measurement of ambient H<sub>2</sub>S concentration

First, it should be noted that at the time of performing the environmental study, there was no noticeable obstructions in any of sewer networks in the previously mentioned regions. Measurement of the ambient H<sub>2</sub>S was done in five areas; inside manhole

opening at a depth of 50 centimeter (2 sewers in Kasr El Aini hospitals), around the openings of the two sewers within a circle of a circumference of 0.5–1 m, and a control area.

Collection of H<sub>2</sub>S gas was accomplished by utilizing the absorption method. The contaminated air was drawn using a calibrated vacuum pump, dry gas meter, and a glass bubbler containing 50 mL of the reagent (2 CdSO<sub>4</sub> · 8 H<sub>2</sub>O and sodium hydroxide). The sampling pump was calibrated to draw 1l/min and air was sampled for 2 hours. This procedure was repeated 5 times at each place of sampling. The methylene blue method was used for (H<sub>2</sub>S) measurement (Harrison and Perry, 1986).

### Statistical analysis

Results were evaluated for each group. Data were compared using Student's *t* test. Qualitative data were compared using chi square test. Analysis of variance (ANOVA) test was used to compare exposed subgroups and control subjects. Pearson correlation test was used to test the correlation between different variables among the exposed groups. The statistical significance was defined as *p* value <0.05. Computer-based statistical package for social sciences (SPSS) for windows 9.1 program was used.

### Results

The mean concentration of H<sub>2</sub>S in the air samples drawn from the inside of manhole opening was 13.2 mg/m<sup>3</sup> (9.4 parts per million [ppm]) with a range (12.2 mg/m<sup>3</sup> – 14.6 mg/m<sup>3</sup> i.e. (8.8–10.5 ppm). There was only one sample in which the H<sub>2</sub>S concentration exceeded the Permissible Exposure Limit (PEL) established by the Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH; 14 mg/m<sup>3</sup> i.e. 10 ppm). Unsurprisingly, much lower concentrations were found in the air samples drawn from the area around the sewer openings, with mean concentration of 6.9 mg/m<sup>3</sup> (4.8 ppm; range = 7.5–9.2 mg/m<sup>3</sup> (5–6.6 ppm). The control area showed nil concentration.

This study was conducted among 63 males divided into an exposed sewer network maintenance workers (*n* = 33) and a matched control group (*n* = 30). The age range of the individuals in the exposed group was (28–57 years), with a mean value of 47.36 ± 8.23 years showing no statistically significant difference when compared with the control group (range =

**Table 1.** Frequency distribution of neurological symptoms among both hydrogen sulfide (H<sub>2</sub>S) exposed (*n* = 33) and control (*n* = 30) groups as detected from the clinical history

Symptoms	Exposed, <i>n</i> = 33		Control, <i>n</i> = 30		<i>p</i>
	<i>N</i>	%	<i>n</i>	%	
Headache	12	36.36	7	23.3	>0.05
Memory defect	12	36	3	10	<0.01 <sup>a</sup>
Lack of concentration	14	42.4	3	10	<0.01 <sup>a</sup>

<sup>a</sup> Statistically significant *p* < 0.01.

25–56 years, mean = 44.18 ± 9.09). The mean duration of working years of the exposed personnel was 17.9 ± 6.68 years (rang = 5–27 years).

As regards neurological symptoms suggestive of cognitive dysfunction (headache, memory defects and lack of concentrations), statistically significant higher frequencies were found among the exposed group compared to their controls (Table 1; *p* < 0.5). Clinical physical and neurologic examinations were unremarkable.

Neurophysiological testing showed significantly prolonged mean values of P300 latency (range = 432–614 msec) and simple reaction time (range = 296–438 msec) in exposed subjects compared to their controls (*p* < 0.001; Table 2) with no significant difference between the two examined groups regarding the P300 amplitude (data not presented). It is worth noting that, there were six exposed workers (18%) whose P300 latency exceeded 500 msec. As regards neuropsychological testing, the sewer workers showed poor performance in most of WMS-R subtests. Apart from the figural memory subtest, the mean scores of the other subtests (visual paired associates I, visual reproduction I and logical memory) were lower among the exposed subjects (Table 2; *p* < 0.01). Among the exposed subjects, there were three (9.5%) workers who had scored <25 in logic memory test.

Qualitative analysis of the results of digit forwards and backwards revealed significant statistical difference between exposed and control groups as regards number of subjects having impaired and defective scores (*p* < 0.05; Table 3)

Verbal fluency and trail making A test were used as indicators for frontal executive functions. Scores of verbal fluency in the exposed workers were significantly lower than the controls (Table 2; *p* < 0.001). Besides, there were 20 out of the 33 exposed workers who scored <15 words (80%) versus only 1 out of

**Table 2.** Mean  $\pm$  SD of the results of neurophysiological tests (simple reaction time [RT] and P300 latency in msec) and neuropsychological tests (Figural memory, visual paired associates I, visual reproduction I, logical memory, and verbal fluency), urinary thiosulfate ( $\mu\text{mol}/\text{mg}$  creatinine) as exposure biomarker, and Mini Mental State Examination test (MMSE) as cognitive screening test in both hydrogen sulfide ( $\text{H}_2\text{S}$ ) exposed ( $n = 33$ ) and control ( $n = 30$ ) groups

	Exposed, $n = 33$	Control, $n = 30$	$p$
Neurophysiological tests			
Simple RT	380.45 $\pm$ 41.43	342.67 $\pm$ 31.51	<0.001
P300 latency	487.32 $\pm$ 45.22	299.14 $\pm$ 20.02	<0.001
Neuropsychological tests			
Figural memory	9 $\pm$ 1.36	8.63 $\pm$ 1.5	>0.05
Visual paired	9 $\pm$ 1.36	10.73 $\pm$ 0.98	<0.001
Visual reproduction	2.55 $\pm$ 0.9	3.9 $\pm$ 0.48	<0.001
Logic memory	29.91 $\pm$ 4	32.33 $\pm$ 2	<0.05
Verbal fluency	11.82 $\pm$ 2.55	16.23 $\pm$ 1.38	<0.001
MMSE	27.33 $\pm$ 1.1	30.0 $\pm$ 0	<0.001
Thiosulfate	50.618 $\pm$ 8.95	25.12 $\pm$ 5.07	<0.001

**Table 3.** Frequency distribution of performance of digit forwards and digit backwards span subtests among hydrogen sulfide ( $\text{H}_2\text{S}$ ) exposed ( $n = 33$ ) and control ( $n = 30$ ) groups

	Normal <sup>a</sup>				Impaired <sup>b</sup>				Defective <sup>c</sup>				$p$
	Exposed		Control		Exposed		Control		Exposed		Control		
	No	%	No	%	No	%	No	%	No	%	No	%	
Digit forward	18	54.5	30	100	12	36.3	0	0	3	9	0	0	<0.05
Digit backward	9	27.2	27	90	15	45.4	3	10	9	27.2	0	0	<0.05

<sup>a</sup> Normal score of digit forwards is  $6 \pm 1$ ; normal score of digit backwards is  $5 \pm 1$ .

<sup>b</sup> Impaired score of digit forwards is 4; impaired score of digit backwards is 3.

<sup>c</sup> Defective score of digit forwards is 3; defective score of digit backwards is 2.

the control subjects (3.33%). Out of the 20 workers scoring <15 words, there were six workers who scored <10.

As regards trail making A test (a qualitative test for attention), 15 exposed subjects (23.8%) failed to perform this test while all control subjects performed this test successfully.

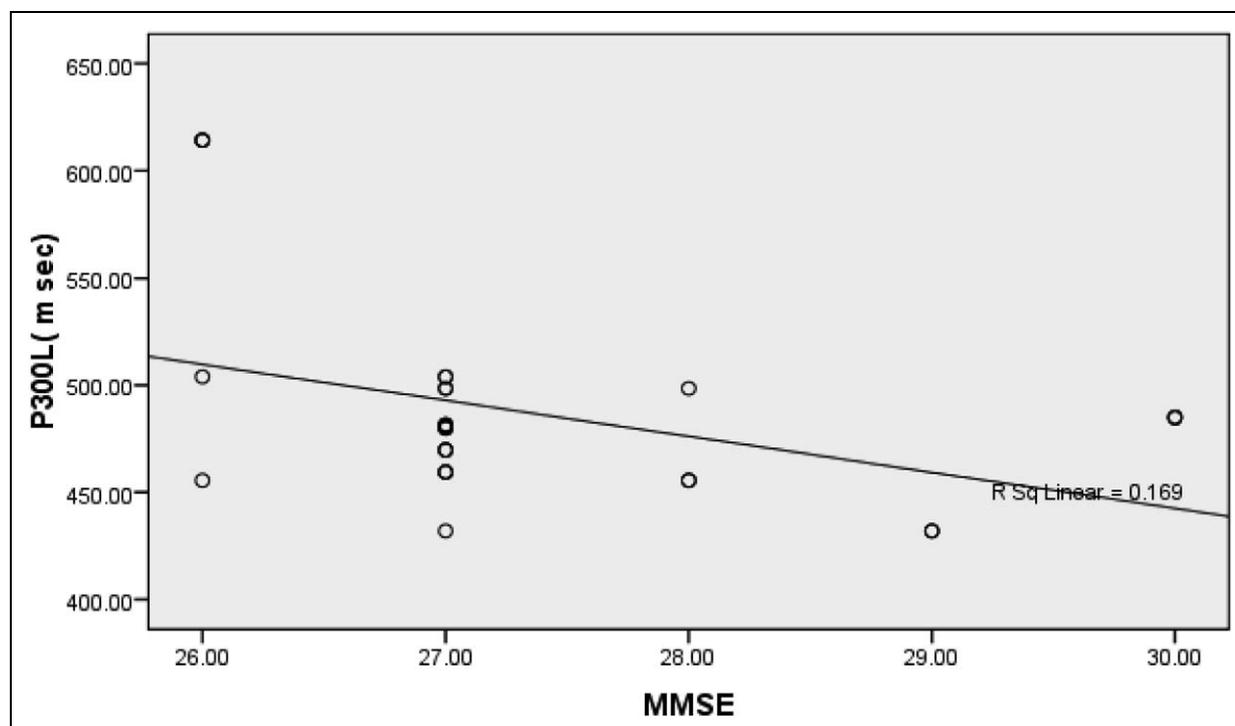
Estimation of urinary thiosulfate as an index of  $\text{H}_2\text{S}$  exposure showed marked increase of its concentration among the exposed workers compared to their controls (Table 2;  $p < .001$ ). However, this increase was not correlated with any of the applied neurophysiological, neuropsychological tests or the duration of work ( $p > .05$ ; data are not presented).

Taking into consideration the educational level and age during assessment of MMSE test among the study groups, 29–30 was considered the expected normal score. However, although none of the exposed workers scored  $\leq 24$ , which is considered a cutoff point for diagnosis of profound cognitive dysfunction, sewer workers had significantly lower mean score than their

controls ( $p < 0.001$ ; Table 2). A statistically significant correlation was found between MMSE and P300 latency ( $r = -.412$ ,  $p < 0.05$ ; Figure 1). Moreover, simple reaction time and logic memory subtest were negatively correlated with MMSE but without significance ( $r = -0.04$ ,  $-0.138$ , respectively,  $p > 0.05$ ). The exposed group was subdivided according to MMSE score into two groups, the first included those with MMSE score  $>27$  ( $n = 9$ ) and the second group included those with MMSE score  $\leq 27$ . ANOVA test between the exposed subgroups and the controls revealed significantly lower mean values of most scores among the exposed group scoring  $\leq 27$  (Table 4). Regarding trail making A test, there were 3 out of 9 workers with MMSE score  $>27$ , who failed to perform the test, versus 12 out of 24 scoring  $\leq 27$ .

## Discussion

This study shows cognitive functions impairment among sewer network workers as revealed from the



**Figure 1.** Significant negative correlation between Mini Mental State Examination (MMSE) score and P300 latency among sewer network workers ( $n = 33$ ;  $r = -.412$ ;  $p = .017$ ).

**Table 4.** Mean  $\pm$  SD of the results of neurophysiological tests (simple reaction time [RT] and P300 latency in msec.) and neuropsychological tests (visual paired associates I, visual reproduction I, logical memory, and verbal fluency) in exposed subgroups (MMSE score  $> 27$  [ $n = 9$ ] and Mini Mental State Examination [MMSE] score  $\leq 27$  [ $n = 24$ ]) and control group ( $n = 30$ )

	Exposed group; MMSE $\leq 27$ ; $n = 24$	Exposed group; MMSE $> 27$ ; $n = 9$	Control; MMSE = 30; $n = 30$	$p$
P300 latency	498.53 $\pm$ 46.73	457.53 $\pm$ 22.99	299.14 $\pm$ 20.02	<0.001
RT	383.12 $\pm$ 45.66	373.33 $\pm$ 28.16	342.6 $\pm$ 31.51	<0.001
Visual paired	8.75 $\pm$ 1.51	9.67 $\pm$ 0.5	10.73 $\pm$ 0.98	<0.001
Visual reproduction	2.75 $\pm$ 0.84	2.0 $\pm$ 0.86	3.83 $\pm$ 0.46	<0.001
Logic memory	27.62 $\pm$ 2.79	32.76 $\pm$ 4.09	32.7 $\pm$ 2.03	<0.001
Verbal fluency	11.88 $\pm$ 2.9	12 $\pm$ 1.73	16.23 $\pm$ 1.38	<0.001

prolonged simple reaction time and P300 latency besides the poor performance in neuropsychological tests applied in this study. Although urinary thiosulfate concentration is elevated among the exposed workers, it is not correlated with results of any of the applied neurophysiological or neuropsychological subtests. This indicates that thiosulfate can be considered only as exposure marker but it cannot be used as screening test of cognitive impairment resulting from H<sub>2</sub>S exposure. Moreover, exposed workers having MMSE score  $< 28$  show significantly lower mean values in most of the applied tests

pointing to the possibility of using MMSE as a rapid screening test for cognitive impairment with taking 27 as a cutoff point.

Sewer network workers are exposed to multitudes of serious chemical and biological hazards. One of these serious chemical hazards is occasional exposure to high concentrations of H<sub>2</sub>S in the sewer pipes (Wild et al., 2006). However, exposure to H<sub>2</sub>S among sewer workers is not limited to fatal concentrations, but lower dose exposure is encountered during their routine work of inspection and maintenance of the sewer networks, especially in frequently opened pipes.

In this study, although environmental measurement of ambient concentration of H<sub>2</sub>S showed levels that did not exceed the recommended levels stated by OSHA and ACGIH (10 mg/m<sup>3</sup> or 10 ppm) except in one occasion, we cannot exclude the occurrence of higher concentrations especially during obstruction and subsequent stagnation of sewage. However, Working Group on the Assessment of Toxic Chemicals (WATCH), in UK, suggested 5 ppm to be the occupational exposure limit (OEL) for H<sub>2</sub>S. The recommendation to review the OELs for H<sub>2</sub>S is because in the mid-1990s, a series of human volunteer studies were published that suggested exposures as low as 10 ppm might cause a shift to anaerobic metabolism under conditions of physical exertion. This leads to a concern for adverse physiological effects at the existing occupational exposure standard (OES) values for H<sub>2</sub>S of 10 ppm (8-hour time-weighted average (TWA); Costigan, 2003).

Mild cognitive impairment has been described as transitional state between normal aging and dementia. It is mainly focused on working memory, conceptual reasoning, speed of information processing, attention, and executive function (Peteresen et al., 2001). In the current study, investigating the frequency of some central nervous system symptoms that may suggest cognitive impairment (memory defects and lack of concentration) revealed statistically significant higher percentage of these symptoms among the exposed group compared to the controls (Table 1,  $p < 0.01$ ). These results are greatly supported by a similar study of Thorn et al. (2002) who reported significantly increased risks for similar symptoms among Sweden sewage workers. Also, similar symptoms were reported among ex-workers and neighboring residents who were exposed to H<sub>2</sub>S and other reduced-sulfur compounds emitted from an oil refinery in concentrations ranged from a low of 10 parts per billion parts per billion (ppb) to 8.8 ppm (Kilburn et al., 1995; Legator et al., 2001).

However, surveys and studies of subjective reported symptoms may be susceptible to response enhancement bias (i.e. an increase in reported symptoms resulting from the fact that respondents are aware of, and sensitized to, the fact that they are exposed) Therefore, modern neurobehavioral methods are used in the assessment of the early effects due to exposure to neurotoxic agents in working and general environment (Albini and Benedetti, 2004).

Cognitive event-related potentials (ERPs) have been widely used in the study of dementive disorders,

including Alzheimer's disease. Of the major waves observed in the ERPs (N200, P300, and Slow Wave), P300 component corresponds to mental processes such as recognition, categorization of stimuli, expectancy, or short-term memory while there are many regions in the brain, especially in the temporal lobe, the parietal lobe, and the hippocampus, which are thought to be responsible for its generation (Bennys et al., 2007).

The numerous clinical P300 studies strongly suggest that this ERP component, elicited by auditory, visual, olfactory, or somatosensory stimuli, may be clinically useful as an index of cognitive function (Papaliagkas et al., 2008). In our study, there was a significant delay in P300 latency among sewer workers versus their controls ( $487.32 \pm 45.22$ ,  $299.14 \pm 20.02$  msec, respectively,  $p < 0.001$ ) besides there were six workers having abnormally prolonged P300 latency pointing to the possibility of cognitive impairment among H<sub>2</sub>S exposed workers. This goes in accordance with Hirsch's study in (2002) that reported delayed P300 latency as residual chronic effect after H<sub>2</sub>S intoxication without loss of consciousness. Unsurprisingly, delayed P300 latency was accompanied with delayed simple reaction time, which is greatly supported by the study of Kilburna (2003) who reported prolonged simple reaction time among 43% of chronically exposed workers to moderate doses of H<sub>2</sub>S.

Currently, one of the most important tools when assessing early cognitive impairment is neuropsychological evaluation where mild cognitive impairment subjects typically record neuropsychological performance between that of healthy older individuals and demented patients (Arñiz and Almkvist, 2003). In our study, most of the examined subjects showed poor performance of tests assessing attention and short-term memory. Besides, the executive functions were found to be impaired in a large percentage of the exposed workers who failed to perform the trail making A test (23.8%) and those who scored <15 (80%) in verbal fluency test. Comparable findings were reported in previous studies that applied similar neuropsychological tests investigations among occupationally and residentially exposed personnel (Kilburn et al., 1995, Kilburn, 1997; Kilburn, 2003; Legator et al., 2001).

The current mechanistic understanding of H<sub>2</sub>S toxicity is incomplete and the patho-physiological mechanisms underlying the effect of H<sub>2</sub>S on normal brain function, particularly, following chronic

exposure have yet to be identified (ATSDR, 2004). The toxicity of H<sub>2</sub>S has been attributed to its ability to inhibit cytochrome oxidase in a similar manner to HCN but recently, it is hypothesized that H<sub>2</sub>S can reduce intracellular-bound ferric iron to form unbound ferrous iron, which activates iron. Additionally, H<sub>2</sub>S can increase the hepatocyte formation of reactive oxygen species (ROS). H<sub>2</sub>S cytotoxicity, therefore, also involves a reactive sulfur species, which depletes GSH and activates oxygen to form ROS (Truong et al., 2006). However, it is suggested that neurobehavioral effects following long-term exposure is accumulative. That is, each exposure results in increased brain damage. The individual damage does not recover, and brain dysfunction continues for years (Kilburn, 2004).

A study of ultrastructural and morphometric characteristics of nerve cells and myelinated fibers in the cerebral cortex revealed activation of protein synthesis in nerve cells after chronic exposure to natural H<sub>2</sub>S-containing gas in low concentrations (10 mg/m<sup>3</sup>) for 2 weeks. After 1 month, the ultrastructure of myelinated fibers was characterized by sectorial loosening and demyelination (Solnyshkova and Shakhlamov, 2002).

What adds to the complexity of H<sub>2</sub>S toxicity is the existence of a functional genetic polymorphism affecting rhodanese or thiosulfate sulfurtransferase (TST) activity. TST is a mitochondrial matrix enzyme that plays roles in the formation of iron-sulfur proteins, the modification of sulfur-containing enzymes, and is also involved in H<sub>2</sub>S detoxification, thus playing role in the individual susceptibility to H<sub>2</sub>S neurotoxicity (Billaut-Laden et al., 2006).

Regarding urinary thiosulfate, the study revealed remarkable increase of its concentrations among the exposed workers compared to their controls (Table 2;  $p < 0.001$ ). This goes in accordance with the study of Kage et al. (1997) when the authors obtained concentrations of urinary thiosulfate 4–14 times higher among H<sub>2</sub>S-exposed workers than the controls. It is worth noting that H<sub>2</sub>S does not accumulate in the body and the urinary thiosulfate levels peak approximately 15 hours after exposure and drop to control levels by 17 hours post-exposure (Milby and Baselt, 1999). Therefore, the increased concentrations of urinary thiosulfate indicates the continuous daily occupational exposure to H<sub>2</sub>S among our exposed workers. However, a quantitative relationship between H<sub>2</sub>S exposure levels and urinary thiosulfate levels has not been established in many studies

(ATSDR, 2004) and this explains the insignificant correlation between the duration of exposure and the concentration level of urinary thiosulfate ( $p > 0.05$ ). This fact, along with absence of statistically significant correlation between the urinary thiosulfate with any of neurophysiological and neuropsychological parameters used in this study, limits the ability of this biomarker to be an estimate of a threshold level above which exposure is to be stopped.

In the current study, MMSE advantages of brevity and ease of administration were applied to verify its use as a screening test for cognitive dysfunction among workers occupationally exposed to H<sub>2</sub>S gas. The significant decrement in the mean value of MMSE scoring in the exposed group ( $27.33 \pm 1.1$ ) versus ( $30 \pm 0$ ) in the controls was associated with significant negative correlation with P300 latency ( $r = -.412$ ,  $p < 0.05$ ; Figure 1), simple reaction time and logic memory ( $p > 0.05$ ). Similar correlations between MMSE scoring and Wechsler Adult intelligence scale and long latency auditory evoked potentials were reported in many previous studies (Crum et al., 1993).

However, although none of the exposed workers scored  $\leq 24$ , which is the cutoff point of diagnosis of cognitive impairment, more comprehensive neurophysiological and neuropsychological assessment of study subjects revealed an evident degree of cognitive impairment particularly among exposed workers having MMSE score  $< 28$  (Table 4). This finding is greatly supported by the study of Missonnier et al. (2005). The authors performed extensive neurophysiological and neuropsychological assessment for a group of subjects with MMSE score between 25 and 28, with subsequent follow-up after 1 year. Results showed that 54% of those subjects revealed significant cognitive decline. These finding go in accordance with other studies that suggest taking MMSE score of 27 as cutoff point to perform more specific evaluation and therefore allow earlier diagnosis (O'Bryant et al., 2008).

## Conclusion

The findings in our study, taken together with previously reported data concerning adverse neurological effects due to chronic exposure to H<sub>2</sub>S gas, mandate the need for continued research with larger scale studies. This is decided of large sector of subjects occupationally exposed to H<sub>2</sub>S gas. In addition, our study recommends the use of MMSE as a periodic rapid

screening test for cognitive dysfunction among exposed workers then performance of more comprehensive study for workers scoring <27.

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