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Effects of Methanol Vapor on Human Neurobehavioral Measures

Mary R. Cook, Fred J. Bergman, Harvey D. Cohen, Mary M. Gerkovich, Charles Graham, Roger K. Harris, and Linda G. Siemann

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Includes the Commentary of the Institute's Health Review Committee

Research Report Number 42

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HEI Statement
HEALTH EFFECTS INSTITUTE

Synopsis of Research Report Number 42

Does Inhalation of Methanol Vapor Affect Human Neurobehavior?

Background

Methanol (wood alcohol) may become an important alternative fuel for motor vehicles in the near future. The 1990 Amendments to the Clean Air Act require that, in areas of the country that have not met specific ambient air quality standards, certain increases must occur in the number of "clean-fuel vehicles." The legislation does not define "clean-fuel vehicle" in terms of fuel composition but in terms of specific performance requirements. However, methanol is specifically listed as a possible "clean alternative fuel." Methanol use in motor vehicles would probably reduce levels of several regulated air pollutants, such as ozone, particulate matter, and carbon monoxide. However, it would probably increase levels of two other pollutants: methanol and formaldehyde. Policymakers need better information on how exposure to low concentrations of these substances might affect human health and function.

Methanol is clearly poisonous at relatively high levels of exposure, but little is known about whether inhalation of low levels of methanol affects the human nervous system. If introduced as an alternative fuel, the most common human exposure to methanol would be from inhaling vapors released into the air from vehicle emissions, during refueling, and from spills. The Health Effects Institute (HEI) sponsored an exploratory study, summarized here, to see whether exposure to methanol vapor might affect the human nervous system, and if so, what would be the best experimental conditions for examining this effect.

Approach

In this pilot study, Dr. Mary Cook and colleagues exposed 12 young male volunteers to either filtered air or methanol vapor (192 parts per million) for 75 minutes. (This concentration of methanol is estimated to approach the highest concentration that individuals might experience from normal use of methanol-fueled vehicles under a worst-case scenario.) The volunteers underwent 20 commonly used tests of sensory, behavioral, and reasoning performance before, during, and after each exposure. To reduce the possible influence of suggestion, neither the chamber operator nor the subjects knew which exposures were to methanol and which were to filtered air.

Results and Implications

Methanol had no detectable effect on the subjects' performance for most tests. However, performance was slightly impaired in one test measuring memory and concentration. In another test, the subjects' brain wave patterns changed slightly in response to light flashes and sounds. The effects observed in this study were minor and within the range of test values for subjects exposed to air. The possibility that these results were due to chance or normal variability is supported by the lack of positive findings in related tests in which similar effects would have been expected. Nevertheless, further research, with a larger number of subjects, is needed to confirm or refute these results and to investigate other important related questions, including: Would exposure to the same or higher concentrations of methanol for longer periods of time affect other populations, such as elderly men and women, and people with compromised vision? Answers to this and other questions will contribute to a sound basis on which regulatory and policy decision-makers can compare the public health benefits of methanol versus gasoline.

This Statement is a summary, prepared by the Health Effects Institute (HEI) and approved by the Board of Directors, of a research project sponsored by HEI from 1988 to 1990. The study was conducted by Dr. Mary R. Cook and associates at the Midwest Research Institute. The complete report (Effects of Methanol Vapor on Human Neurobehavioral Measures, Research Report Number 42) can be obtained from the Health Effects Institute, 141 Portland Street, Suite 7300, Cambridge, MA 02139 (Telephone: 617-621-0266). The Research Report contains both a detailed Investigators' Report and a Commentary on the study by the Institute's Health Review Committee.

TABLE OF CONTENTS

Research Report Number 42

Effects of Methanol Vapor on Human Neurobehavioral Measures

INVESTIGATORS' REPORT Mary R. Cook, Fred J. Bergman, Harvey D. Cohen, Mary M. Gerkovich, Charles Graham, Roger K. Harris, and Linda G. Siemann

HEALTH REVIEW COMMITTEE'S COMMENTARY Health Effects Institute

Effects of Methanol Vapor on Human Neurobehavioral Measures

Mary R. Cook[1], Fred J. Bergman, Harvey D. Cohen, Mary M. Gerkovich, Charles Graham, Roger K. Harris, and Linda G. Siemann

ABSTRACT

Methanol could become an important motor fuel. The objective of this exploratory study was to provide preliminary information about whether or not acute exposure to methanol at 250 mg/m^3 for 75 minutes would have adverse effects on human neurobehavioral functions important in everyday life. This concentration level was selected because it is at the upper limit of the traffic scenario estimates provided by the U.S. Environmental Protection Agency (EPA)[2] (86 to 240 mg/m^3), but is below the maximum concentrations for eight-hour average exposures currently recommended by the National Institute of Occupational Safety and Health (NIOSH) and the American Conference of Government and Industrial Hygienists (ACGIH) (260 mg/m^3). Although traffic scenario exposure estimates suggest that such high levels of exposure last less than 15 minutes, we used a 75-minute exposure to increase the probability of identifying dependent measures that should be studied in more detail in a future confirmatory study.

Twelve healthy young men, each serving as his own control, participated in two sham exposures and two methanol exposures under counterbalanced, double-blind control conditions. Because methanol is present in many foods, and because high preexposure levels of methanol or formate might obscure exposure effects, subjects were required to strictly limit their diets for 12 hours before each experimental session. The following endpoints were examined before, during, and after exposure to methanol and sham vapors: blood and urinary methanol; plasma formate; oral temperature; blood pressure; subjective mood, alertness, fatigue, workload, and symptom scales; spectral analysis of the electroencephalogram; visual- and auditory-event-related potentials; contingent negative variation; respiration; cardiac interbeat interval; Symbol Digit substitution task; three-choice reaction time; Stroop color-word test; simple reaction time; visual function; critical flicker fusion frequency; hand steadiness; visual search task; Gamberale reaction time task; visual tracking task; Sternberg memory task; interval production task; and speeded addition task. Two dual tasks were also included in the task batteries. These endpoints were selected from those indicated in the literature to be sensitive to solvents, and from those that are widely used in other neurobehavioral test batteries to identify the effects of environmental pollutants. Because the number of endpoints examined was large and the number of subjects was small, procedures designed to reduce the number of statistical tests performed were used.

Mean methanol concentration in the exposure room during methanol exposures was 249 mg/m^3 (SD $\pm$ 7 mg/m^3). Exposure produced significant increases in blood and urine methanol concentration. As expected, no changes in plasma formate were observed.

Most of the neurobehavioral endpoints were unaffected by exposure to methanol; however, statistically significant effects and trends were found for a cluster of variables, including the latency of the P200 component of event-related potentials, performance on the Sternberg memory task, and subjective measures of fatigue and concentration. The effects were small and did not exceed the normal range. These findings might, of course, be spurious, as a great many endpoints were examined on a small number of subjects. The tests were conducted on healthy young male college students, who might be expected to be less sensitive to methanol exposure than other populations. It is, therefore, important to perform further research to verify and extend the results reported here, and to further examine the implications of these results for risk assessment. Such research should employ a larger sample size, systematically vary both concentration of methanol and the duration of exposure, use odor-masking techniques to reduce the probability that either subjects or experimenters will be able to detect the presence of methanol, and include more difficult tasks to determine whether or not effects are in part a function of the difficulty or complexity of the task. Direct comparisons of the effects of exposure to methanol with the effects of exposure to vapors produced by other motor fuels would also be of value.

INTRODUCTION

Within the next 20 years, methanol may be used as fuel for an increasing proportion of the motor vehicles in this

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[2] A list of abbreviations appears at the end of this report for your reference.

country. The concentration of methanol to which the public might be exposed has been calculated for a variety of scenarios, from urban street and highway driving to malfunctions in closed, private garages. These estimates suggest that exposure will be below the currently recommended occupational exposure limits, even for the most severe of the scenarios (roadway tunnel, warmup or hot soak evaporative emissions in private garages, refueling, etc.). The low exposure levels anticipated (less than 250 mg/m^3 for less than 15 minutes) suggest that there will be little risk of morbidity and mortality from the inhalation of methanol vapors.

Estimates of morbidity and mortality alone, however, do not provide sufficient information for risk assessment. Information about effects on memory, performance, cognition, neurophysiology, and other human functions important in daily living is also needed. The methanol literature relevant to these functions is limited (Carson et al. 1981, 1987), and there is a need to develop an adequate data base from which to evaluate whether or not the widespread use of methanol as a motor vehicle fuel would result in any risk to the population of adverse neurobehavioral effects.

Neurobehavioral effects cannot be considered trivial. The ability to perceive the world around us, to process information quickly and accurately, to respond effectively to environmental and occupational demands, and to maintain attention and arousal are important to highway safety and to survival in general. Previous work has shown that laboratory studies of subtle neurobehavioral effects are predictive of performance in the workplace, and of the ability to perform complex, real-world tasks such as driving and flying (for example, O'Donnell and Hartman 1979; Alluisi and Fleishman 1982). Even when performance levels can be maintained, the increased workload required under exposure to some pollutants can increase fatigue and stress levels and thereby indirectly affect morbidity and mortality. Thus, the purpose of this preliminary investigation was to determine whether or not exposure to low concentrations of methanol vapor resulted in neurobehavioral effects warranting further study.

SPECIFIC AIMS

Our major aim was to perform a controlled preliminary screening study of the effects of exposure to methanol vapor at 250 mg/m^3 on multiple measures of human visual, behavioral, cognitive, and physiological functions important in daily living. A secondary aim was to determine if this low level of exposure would have a measurable impact on the levels of methanol in the blood and urine of the volunteers, and if so, to evaluate if such changes were correlated with changes in neurobehavioral variables. Formate is a major metabolite of methanol, and it is believed to be responsible for the visual consequences of methanol ingestion. Blood formate concentrations were, therefore, analyzed to determine whether or not any unexpected changes could be observed during the brief experimental sessions; if changes in formate did occur, it would be necessary to examine whether or not any neurobehavioral changes could be explained by changes in formate concentrations, rather than changes in the concentration of methanol itself in body fluids.

Because so little information was available from previous research, only three specific hypotheses were established: (1) methanol concentrations in blood and urine will be higher immediately after exposure to methanol vapors than after sham exposure; (2) methanol concentrations in urine will not return to base-line levels by the end of the experimental session; and (3) no detectable changes in formate concentration will be seen during the time period involved in the experimental session.

With regard to neurobehavioral measures, the study reported here was exploratory in nature and was designed to provide guidance for future, more detailed investigations of the effects of methanol inhalation on humans.

METHODS AND STUDY DESIGN

APPROACH TO THE PROBLEM

Using human subjects to study the effects of methanol provides directly relevant data without the problems of extrapolation from another species. Laboratory, rather than occupational, exposure was selected because exposure levels could be set in the range relevant to the use of methanol as a motor fuel, base-line measures could be obtained before exposure, each subject could be used as his own control, and collection of data on the dependent measures would not be subject to the time and logistic restrictions inherent in occupational field studies.

The next question addressed in planning the research was the level of exposure to be used. The EPA (Health Effects Institute 1987) had estimated that the highest vehicle-related exposure to methanol vapors would occur in a closed garage with poor ventilation after the engine was turned off. The estimated level was 86 to 240 mg/m^3. We, therefore, selected an exposure level of 250 mg/m^3, because it was slightly above the highest exposure estimate provided by the EPA, and slightly below the maximum concentra-

tions for an eight-hour time-weighted average recommended by NIOSH and ACGIH (260 mg/m^3).

It was important that neurobehavioral testing be carried out when the body burden of methanol had reached a steady state. Benoit and associates (1985) reported that a steady state of methanol in expired air was reached in about 30 minutes when subjects were exposed to 150 to 160 mg/m^3. They also reported that the half-life was approximately 24 minutes, although there were marked differences between subjects in this regard. The half-life in blood and urine is approximately 2.5 to 3 hours (Leaf and Zatman 1952; Sedivec et al. 1981; Stegink et al. 1981, 1983). These considerations, together with the material presented in the Health Effects Institute Special Report (1987), suggested that subjects should be exposed to methanol vapor for at least 30 minutes before neurobehavioral testing.

A relatively homogeneous sample of healthy, nonsmoking young men was selected to increase precision, reduce the sample size required, and maximize the probability of detecting any existing effects by reducing between-subjects variability. Because the men were in good health, the probability of adverse reactions to exposure was also reduced. Because there was not enough information available about the neurobehavioral effects of methanol to conduct power analysis (Cohen 1977), sample size was based on our previous experience with the dependent variables to be used. The variances obtained in previous repeated measures studies in our laboratory that employed the same endpoints were used to calculate required sample size. Data were available for electroencephalogram (EEG) spectral analyses, auditory- and visual-event-related potentials, cardiac interbeat interval, critical flicker fusion, simple and choice reaction time, Symbol Digit substitution, interval production task, Gamberale reaction time task, and two-digit speeded addition. Conservative assumptions were made. We assumed that any effect observed would be small, and that the correlation between exposed and nonexposed conditions (each subject as his own control) would be approximately $r = 0.80$. Under these assumptions, a sample size of nine individuals would, for most of the dependent measures, provide 80 percent probability of detecting a 10 percent change at the $p = 0.05$ level if, in fact, an effect existed. Because so many assumptions were made, a sample size of 12 was selected.

Simply knowing that one is being exposed to methanol vapor can affect neurobehavioral test results. Therefore, a double-blind study design was used. Measures to evaluate the efficacy of the double-blind procedures were included to assist in data interpretation. Because data on thresholds for human perception of the odor of methanol vary widely, a preliminary study was conducted to determine whether or not subjects would be likely to detect the odor of methanol under the particular exposure conditions to be used. The preliminary study, described in detail in Appendix A, indicated that subjects were unable to detect methanol at better than chance levels.

The experiment was designed to evaluate the effects of brief, low-level exposure to methanol. Any neurobehavioral effects found under such conditions would, therefore, be expected to be small and subtle. Because so few data about the effects of methanol exposure on human function exist, the experiment was exploratory in nature. The neurobehavioral test battery was designed to screen a wide variety of functions important in daily life. Because so many endpoints were studied, it was necessary to divide the battery into two parts. When possible, test items were selected from well known neurobehavioral toxicology batteries that are sensitive to the effects of exposure to solvents and other environmental pollutants (see Neurobehavioral Testing Procedures section for details). So that both improvements and decrements in performance could be measured, subjects were trained to adequate, but not overlearned, levels of proficiency on the performance tasks in the battery. This strategy also allowed us to evaluate the effects of exposure on such factors as practice and fatigue.

EXPERIMENTAL DESIGN

Twelve healthy young men participated in two training sessions and four experimental sessions. All experimental sessions were performed in the morning and, for a given subject, all began at the same time of day. At random, half of the subjects were first trained and tested on battery A, and then trained and tested on battery B (AABB); the other half was first trained and tested on battery B (BBAA). Within each of these groups, half the subjects, selected at random, were exposed in the order sham-methanol-sham-methanol, and half in the order methanol-sham-methanol-sham. Each subject, therefore, served as his own control. During methanol exposures, the subjects were exposed to methanol vapor at 250 mg/m^3 for 75 minutes. During sham exposures, exposure was to filtered room air. Battery A focused on the measurement of physiological activity and included few performance tasks. All measures were obtained before, during, and after exposure. Battery B assessed more complex cognitive and psychomotor functions, and included the performance of dual tasks. Measures in this battery were obtained before, during, and after exposure, with the following exceptions. Three tasks in battery B (visual function, hand steadiness, and critical flicker fusion) were designed to be administered by the experimenter. They could not be performed by the subject while he was alone in the test

room during the exposure. The "during exposure" data points for these measures were obtained approximately 15 minutes after the exposure ended.

Blood and urine samples for quantification of methanol and formate levels were taken before and immediately after exposure; a third urine sample was obtained at the end of each experimental session. Because measurable levels of carbon monoxide were observed in the laboratory early in the study, carbon monoxide levels of subjects' expired air were also determined before and after each experimental session.

STUDY POPULATION

Midwest Research Institute (MRI) has established a Human Subjects Committee in accordance with U.S. Department of Health and Human Services regulations on Protection of Human Subjects (45 CFR 46 as amended). This committee reviewed the proposal for the work reported here, approved all project activity plans and consent forms, and maintained continuing review of the program during the data collection period.

Notices describing the research program were posted at local colleges and universities. Thirty-four potential volunteers called and were given a complete and accurate description of the goals, procedures, risks, and benefits of participation. Those interested in participation in the study were asked a series of questions to determine whether or not they were eligible to participate (see Eligibility of Experimental Subjects section for details). Those who met the criteria were personally interviewed by the Principal Investigator. The study was explained again, the volunteer was shown the facility, and written informed consent was obtained. After this interview, one man was dropped before the physical examination when he reported that he was allergic to ethanol; one decided not to participate; and two were eliminated because of health problems discovered during the physical examination. The medical history and physical examination conducted in the physician's office evaluated respiratory disorders, cardiac function, liver function, kidney function, eye disorders, and history of allergies and drug or alcohol abuse.

The remaining 12 paid volunteers completed all study requirements. The mean age of the group was 26 (range 22 to 32); mean height was 71 inches (range 66 to 79), and mean weight was 169 pounds (range 140 to 280). Five were undergraduate students at a local university, two were graduate students, three were dental students, and two were medical students.

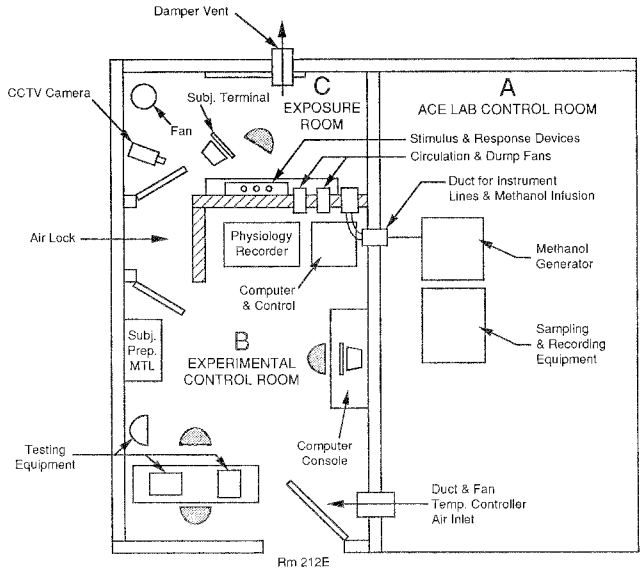


Figure 1. Test facility for examining the effects of exposure to methanol vapor on human function. CCTV = closed-circuit television. MTL = materials. Scale: 1/4 in = 1 ft.

TEST FACILITY AND APPARATUS FOR NEUROBEHAVIORAL TESTING

A human exposure test facility was designed, constructed, and equipped for the experiment. As shown in Figure 1, the facility consists of three rooms. Room A is the control room, where the generation and monitoring of methanol and sham vapor took place. The procedures used for this purpose are described below in the Exposure System and Dosimetry section.

Rooms B and C were constructed specifically for human exposure testing. Room B was used for general control of the test battery. Rooms B and C were equipped with closed-circuit television and audio intercom systems to allow continuous monitoring of and communication with the volunteer when he was in the exposure and test room (C). Electrophysiological measurement equipment included an 8-channel, Beckman (Fullerton, CA) Type RM recorder interfaced to a Digital Equipment Corporation (Merrimack, NH) PDP 11/23 minicomputer. The recorder produced a paper record, and passed physiological signals to a 12-bit analog-to-digital (A/D) converter residing in the data collection computer. The three experimenter-administered tasks included in battery B (visual function, hand steadiness, and critical flicker fusion) were performed using equipment located in room B.

The test room (room C) was connected to the adjacent control room (B) by a double-door passageway that served as an air lock to minimize disturbance of the air:methanol concentration. The methanol concentration monitoring equipment and purge fans installed in the test room were

controlled directly from room A. The test room was equipped with a comfortable chair, a computer terminal, and various stimulus-response equipment to perform the test batteries (stereo headphones, diode stimulus device emitting red or green light, three-button response box, joystick, and loudspeaker). Stimulus-response devices were interfaced to the computer via a Med Associates (East Fairfield, VT) logic system. The voice stimuli used in various battery tasks were generated by a Model 600 Cognitronics (Stamford, CT) voice generator located in the control room.

A software system controlled the overall administration of the test batteries. It allowed the experimenter to select any of the battery tasks for presentation and to control on his terminal the administration of the computer-interactive tasks the volunteer performed. Tasks were presented according to a standardized protocol.

EXPOSURE SYSTEM AND DOSIMETRY

The test room had a volume of 17.24 m^3 and the air was exchanged 3.45 times per hour, so that the air exchange rate was 59.5 m^3/hour. This required a system capable of generating methanol vapor at 11.9 g/hour. Vapor generation was accomplished using a glass container suspended in a temperature-controlled water bath. The heated container was fed ultra-high-purity methanol (Burdick & Jackson, Muskegon, MI) using a syringe pump. High-purity air metered with a precision rotameter was directed through the heated methanol container and added to room air.

Once neurobehavioral data had been obtained before exposure, the subject left the test room and the methanol level was rapidly raised to the test level. This was accomplished by rapid stirring of air within the test room and the rapid injection of 5 mL of methanol into the vaporizing system to bring the room concentration close to the test level, as indicated by a MIRAN® (Foxboro Analytical, South Norwalk, CT) infrared analyzer. The concentration was then trimmed to the exact test concentration and maintained at that level, using the methanol vaporizing system. The procedure usually required less than 10 minutes. The subject then returned to the test room. Monitoring of room concentration using the MIRAN® and a backup automated gas chromatographic system continued throughout testing. Quality assurance procedures are described in detail in the Exposure section under Quality Assurance Procedures.

At the end of the exposure, the subject again left the test room, the methanol vaporization system was shut off, and high-volume exhaust fans were activated. The concentration was reduced to a few percent of the original value within 10 minutes, and usually approached nondetectable limits within 15 minutes. To verify the removal of methanol from the test room before each sham exposure, the output from the continuous analyzers was examined. Tests were postponed if background levels were more than 5 parts per million (ppm).

To maintain the double-blind control of the experiment, all operations were the same on sham exposure days as on methanol exposure days, except that filtered room air was introduced into the vaporizing system instead of methanol. Wet and dry bulb temperature measurements and barometric pressure measurements were obtained automatically at a sampling rate of once per minute. Carbon monoxide concentrations in the test room were determined before and after each experimental session.

NEUROBEHAVIORAL TESTING PROCEDURES

Because the number of dependent variables to be examined in the experiment was so large, they were divided into two test batteries. Battery A focused on psychophysiological measures and simple cognitive or performance tasks, and battery B focused on cognitive, perceptual, and psychomotor functions. Subjects were trained according to previously established criteria on each battery item during training sessions completed before the experimental sessions in which the battery would be used. Methanol exposures were separated by 6 to 14 days (mean 11 days), depending on the subject's schedule. All neurobehavioral training and test batteries were administered by the same individual.

Procedures Used in All Experimental Sessions

Every attempt was made to prevent extraneous sources of stress from affecting study results. Experimental sessions were scheduled at times when the subject did not anticipate any major stressors. If the subject became ill, or reported to the laboratory sleep-deprived or with symptoms of illness, the session was rescheduled. Magazines of general interest (for example, *Smithsonian* and *National Geographic*) were provided, and the subject was not allowed to bring his own reading material.

The literature suggests that levels of methanol (for example, Ferry et al. 1980; Sedivec et al. 1981; Liesivuori and Savolainen 1987) and formate (for example, Boeniger 1987) in body fluids vary widely both between and within individuals. In an attempt to reduce the impact of this variability on study results, the following steps were taken: each subject served as his own control; subjects eliminated alcoholic beverages, diet foods and drinks, fruits and fruit juices, and coffee from their diets for at least 12 hours before each experimental session; and subjects were given a standardized breakfast. Each subject chose from a limited menu,

and then received his choice before each of the four experimental sessions. During the 24 hours before each experimental session, the subject maintained a log of all foods and beverages consumed. The log was collected by the experimenter at the beginning of each session and reviewed with the subject to emphasize the importance of complying with the dietary limitations of the study.

Ten measures were obtained in all experimental sessions. The Health Effects Questionnaire (Appendix D) addresses all symptoms and complaints identified in the literature as being associated with exposure to methanol vapor, as well as other common complaints. It was administered at the beginning and end of each experimental session. The Mood Adjective Check List (Nowlis 1970), which provides 11 mood scales, was administered before and after the exposure, and again at the end of the experimental session. A scale developed in our laboratory was used to measure subjective mental alertness, and scales developed by the United States Air Force School of Aerospace Medicine (Pearson and Byars 1956) were used to measure subjective fatigue and workload. These measures, which are shown in Appendix D, were also obtained before and after exposure, and at the end of the experimental session.

At the end of each exposure, the subject and the experimenter who administered the neurobehavioral test batteries used separate forms (see Appendix D) to judge independently whether or not methanol vapor was present during the exposure. They also rated their confidence in the judgments and listed the reasons for their decisions.

Oral temperature, pulse rate, and systolic and diastolic blood pressure were determined at the beginning and end of each experimental session and immediately after the exposure.

Measures Included Only in Battery A

Table 1 lists the physiological and performance measures obtained in battery A. The measures are shown in the order in which the tasks were administered, and the time required for each task is included. In the following discussion, rationale for task selection is discussed first, followed by details of task administration procedures.

Battery A included four measures derived from the EEG: spectral analysis, auditory- and visual-event-related potentials, and the contingent negative variation. Spectral measures by Fast Fourier Transform (FFT) of ongoing, spontaneous EEG activity provide an index of overall central nervous system activity and are useful in characterizing changes in the level of physiological arousal (Arezzo et al. 1985). Such measures have been used previously in studies of automobile driving behavior (O'Hanlon and Kelley 1974) and as an index of the effects of exposure to ethanol (for example, Doctor et al. 1966; Knott and Venables 1979), carbon monoxide (O'Donnell et al. 1971), and organic solvents (Iregren 1986).

Table 1. Neurobehavioral Task Battery A

Test Sequence	Time to Perform (minutes)
Physiological base line	3
Visual-event-related potential (2-minute rest break)	6
Auditory-event-related potential (2-minute rest break)	6
Symbol Digit substitution task	2
Choice reaction time task	1
Stroop color-word test (1-minute rest break)	2
Contingent negative variation	4

Presentation of a stimulus will evoke transient electrophysiological responses in the human brain. Computer-averaging of such event-related potentials (also called evoked responses) recorded at the scalp results in a complex EEG waveform with distinct, electrically positive and negative components. Changes in the early components of the waveform (those that occur within 80 milliseconds after stimulus onset) primarily reflect sensory processing, and have been widely used in neurotoxicological studies to assess the integrity of sensory systems (Arezzo et al. 1985). Changes in the early, sensory components have been observed in response to exposure to ethanol, carbon monoxide, xylene, methylene chloride, and trichloroethylene (Dick and Johnson 1986).

Changes in the later components of the waveform primarily reflect cognitive processes. A large body of research indicates that analysis of changes in the latency and amplitude of these components can provide meaningful information on the neurophysiological correlates of complex cognitive function (Bodis-Wollner 1982; Chiappa and Roper 1982; Hillyard and Kutas 1983; Karrer et al. 1984). At present, late-component measures are more appropriate for use in controlled laboratory studies than as indices of toxicant exposure in the testing of large populations (Harbin 1985; Otto et al. 1985). In the laboratory, changes in the later components of event-related potentials have been shown to be affected by exposure to ethanol (Zilm 1981), to nicotine (Michel et al. 1987), and to lead and some solvents (for example, Dick and Johnson 1986). Both visual- and auditory-event-related potentials were measured in battery A. These measures were included to provide preliminary information about the influence of exposure to methanol vapor on the neurophysiological correlates of higher-order cognitive activities. Event-related potential measures were obtained

in the context of the Donchin Oddball paradigm (Donchin 1981). This is the most widely studied and best standardized of the available paradigms (Harbin 1985; Otto et al. 1985) to evaluate the response of the brain to visual and auditory stimuli. The early components reflect sensory processes, and the later components reflect cognitive processes.

The contingent negative variation (Rockstroh et al. 1982) is frequently used in laboratory studies, but has not been widely used in neurotoxicological research. It was included in the battery because of its sensitivity to moment-to-moment changes in attention (McCallum 1969), to motivational level (Irwin 1966), and to reaction time (Norton and Howard 1988). The measure is altered by ethanol (Knott and Venables 1980) and by very small doses of nicotine (for example, Ashton et al. 1978).

Cardiac interbeat interval and respiration were monitored during battery A to determine whether or not they were affected by methanol exposure. In addition, averaged electrocardiogram (ECG) measures were obtained to aid in the interpretation of any changes observed in interbeat interval. Heart rate and respiration have both been shown to be sensitive to a wide variety of environmental and subjective factors (O'Donnell and Hartman 1979; Martin and Venables 1980).

The Symbol Digit substitution task has been included in six major neurobehavioral test batteries (Gullion and Eckerman 1986) as a measure of attention and information processing. The choice reaction time task, which is also included in many neurotoxicological test batteries, has been shown to be sensitive to methyl chloroform, toluene, styrene, and xylene (Gamberale and Hultengren 1972, 1973, 1974; Seppalainen et al. 1981), to ethanol (Perrine 1976), and to a wide variety of situational factors. The Stroop color-word test measures the degree to which an individual's ability to focus attention is susceptible to interference (Stroop 1935). The task is included in the Microtox battery and as an additional test in the World Health Organization (WHO) battery (Gullion and Eckerman 1986); it has been shown to be affected by ethanol (Iregren 1986).

Administration of the battery always began with the measurement of physiological activity under resting conditions. During the physiological base-line period, the subject was instructed to sit quietly and comfortably with his eyes closed. Recordings of brain, eye, heart, and respiratory activity were obtained during this period.

Brain (EEG) activity was recorded from standard vertex and parietal scalp sites (Cz and Pz in the international 10–20 system; Jasper 1958) using biopotential electrodes referenced to linked mastoids. The recording sites were first prepared by cleaning them with isopropyl alcohol and then lightly abrading them to reduce electrical resistance below 5,000 ohms. The biopotential electrodes were filled with Beckman electrode paste to enhance electrical skin contact, and then held against the scalp using 2-×-2-inch gauze pads impregnated with EEG electrode cream (Type EC2, Grass Medical Instruments, Quincy, MA). Eye (electro-oculogram [EOG]) activity for eye artifact rejection was recorded using small-diameter biopotential electrodes filled with Beckman electrode paste. The electrodes were attached with double-stick adhesive collars to prepared supraorbital and lateral sites around the left eye.

Electroencephalogram activity for spectral analysis was collected for four four-second intervals starting at the beginning of the third minute. If an eye blink occurred during any of the four-second collection intervals, the EEG data collected in the entire interval were discarded and replaced. Data from the vertex and parietal sites were sampled at a rate of 128 samples/second. Fast Fourier Transform (FFT) analysis using a Hanning window (Cooley and Tukey 1965) was performed off-line on the raw EEG data. Percent energy, peak frequency, peak frequency amplitude, and power in the theta (4.5 to 7.5 Hz), alpha (8 to 12 Hz), and beta (12.5 to 26 Hz) bands, as well as overall power and dominant frequency, were calculated by the software.

Cardiac activity was recorded using silver–silver chloride biopotential electrodes attached with double-stick adhesive collars to prepared skin sites on the right clavicle and the seventh intercostal space under the left ancillary midline (lead II). Beckman electrode paste served as the contact medium. Cardiac signals were amplified and digitized at 256 samples/second; the signal was also processed by a Beckman 9857 cardiotachometer coupler to produce a paper record of heart rate. The coupler also passed a pulse, generated by the R wave, to a digitizing channel. Using the information provided by the two A/D channels, software developed in our laboratory was used to determine the amplitude and latency of each of the components of the cardiogram as well as the cardiac interbeat interval.

Thoracic and abdominal respiration were recorded using mercury capillary-length gauges placed around the body just under the armpits and at the level of the umbilicus. The gauges acted as elastic variable resistors; changes in length as a function of respiratory movements were converted to voltage changes, conditioned through Beckman Model 9875 mercury gauge couplers, and displayed on the Beckman chart recorder.

Both visual- and auditory-event-related potentials were obtained during performance of the Oddball task (Donchin 1981). The Oddball task is widely used in cognitive psychophysiology to evaluate the response of the brain to the detection of infrequent stimuli. The visual-event-related

potential was obtained in response to a red- or green-light-emitting diode stimulus device located directly in front of the subject. Each stimulus flash of the diode was 50 milliseconds in duration. A randomized series of 140 red and green stimuli was presented at approximately two-second intervals, with green flashes defined as common (nontarget stimuli) and red flashes defined as rare (target stimuli, 15 to 18 percent of the series). The subject's task was to press a response button as soon as he detected a red flash, and to maintain a running count of the total number of red flashes presented. The auditory-event-related potential task was similar, except that distinctive low-pitched tones (nontarget) and high-pitched tones (target, 15 to 18 percent of the series) presented through stereo headphones were used as stimuli. The tones were presented for 50 milliseconds, with a rise-and-fall time of 10 milliseconds. During this task, subjects were instructed to keep their eyes closed.

Behavioral data for the auditory- and visual-event-related potential tasks include reaction time to each target stimulus and the accuracy of the subject's count. Physiological data include measures of the average response recorded from the vertex to linked mastoids (sampling rate = 100 Hz; data epoch = 100 msec base line, 700 msec poststimulus collection period; time constant = 0.3 seconds; high-frequency cutoff = 32 Hz). Parietal recordings were also made to aid in data interpretation. These data were used only to confirm the latency of the components of the vertex event-related potentials and were not included in the analyzed data. Electrooculogram data were recorded simultaneously from supraorbital and lateral sites, and were used to delete eyeblink and eye-movement artifact data from the EEG averages. Figures 2 and 3 show sample auditory and visual waveforms to target and nontarget stimuli.

The Symbol Digit substitution task, based on the standard subtest of the Wechsler adult intelligence scale, provides a measure of human information processing abilities under time pressure. The task was modified so that it could be presented and automatically scored using the PDP 11/23 computer. During testing, a code list associating different symbols with the numbers one through nine appeared at the top of a computer screen. One of the code symbols appeared in the center of the screen. The subject's task was to identify the number associated with the single symbol, and then to enter the number into the computer. The task lasted

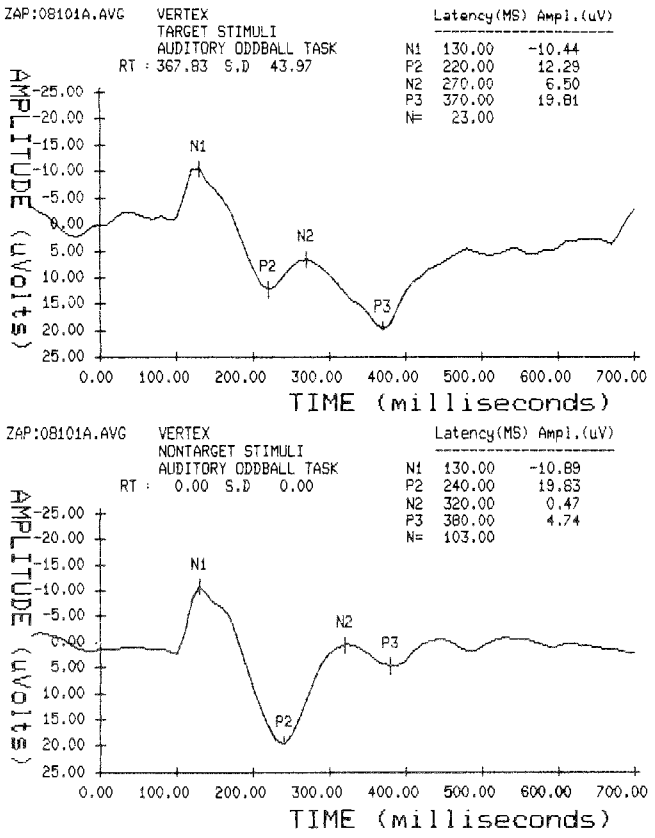


Figure 2. Sample auditory-event-related potential waveforms to target and nontarget stimuli.

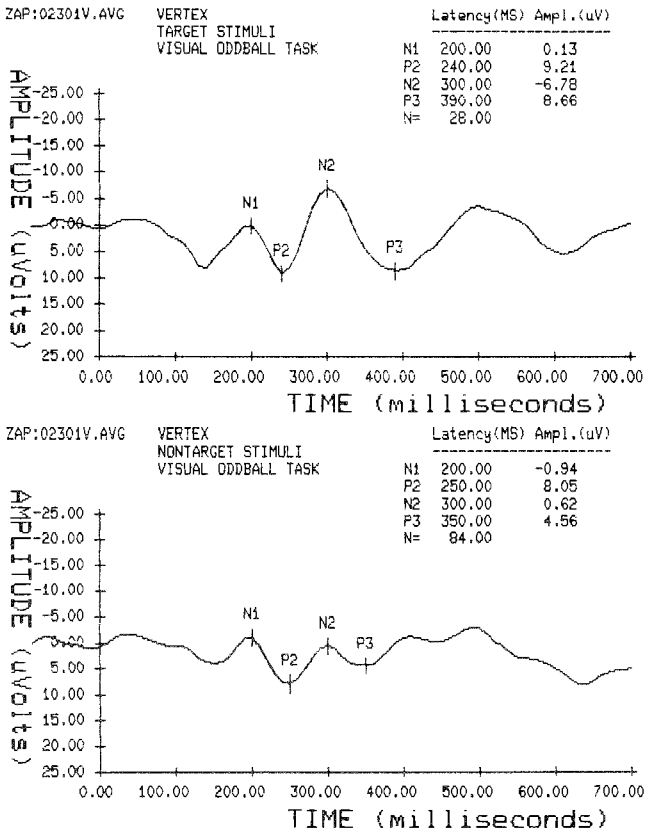


Figure 3. Sample visual-event-related potential waveforms to target and nontarget stimuli.

90 seconds, and the measures used were the number completed and the number correct.

The choice reaction time task measures the speed of low-level decision-making under time pressure. The subject saw a display consisting of three boxes, one of which contained an X. The boxes corresponded to keys on the computer keyboard, and the subject's task was to press the correct key as quickly as possible. One hundred stimuli were presented, and both mean reaction time and accuracy were measured.

The Stroop color-word test (Stroop 1935) measures the degree to which an individual's ability to focus attention is susceptible to interference. The task consisted of two parts. In part 1, the subject's task was to identify the color of a bar of asterisks by printing the first letter of the color's name under the bar. One minute was allowed to complete as many bars as possible. Part 2 consisted of rows of color names, but each name was printed in inks of different colors. The subject's task was to ignore the word he saw, and focus on the color in which it was printed. Again, one minute was allowed. Performance measures were number completed and number correct for each part.

The contingent negative variation occurs when several pairings of stimuli have been made using a constant time period between a "warning" stimulus and an "imperative" stimulus. The subject presses a button as quickly as possible after the imperative stimulus. Twenty artifact-free trials were averaged to obtain the contingent negative variation. Each trial consisted of a high-pitched warning tone, followed two seconds later by a low-pitched imperative stimulus. A random intertrial interval (range 9 to 14 seconds) was used. Data recorded from the vertex were digitized at 100 Hz; the time constant was 30 seconds. Measures included reaction time, maximum contingent negative variation deflection, latency of onset of the contingent negative variation, and latency of the maximum deflection. Figure 4 shows a typical waveform.

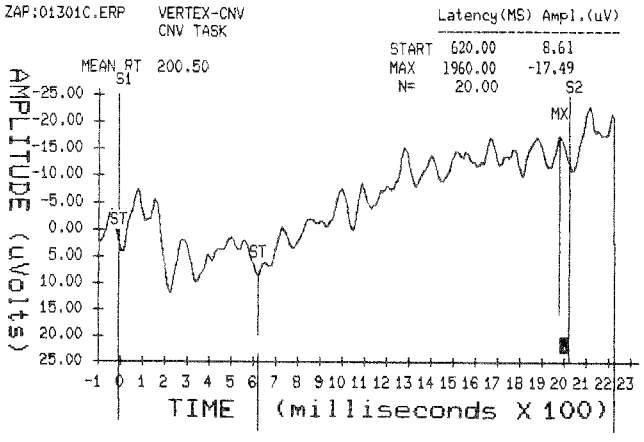


Figure 4. Sample contingent negative variation waveform.

Table 2 summarizes the procedures for experimental sessions and Figure 5 shows the time line for battery A.

Measures Included in Battery B

Table 3 lists the measures obtained in battery B in the order in which the tasks were administered; the time required for each task is included. In the following discussion, rationale for task selection is discussed first, followed by details of task administration procedures.

Table 2. Procedures for Experimental Sessions

Preexposure Activities
- The 24-hour diet log is collected and vital signs measured.
- The subject eats breakfast and completes the self-report scales.
- The subject performs the preexposure task battery (automated tasks in the exposure room and nonautomated tasks in the control room).[a]
- Blood and urine samples are collected.
- Methanol or sham vapor is introduced into the exposure room.

Exposure Activities
- The subject is exposed for 75 minutes to either methanol (250 mg/m^3) or sham vapor.
- He is monitored via TV and audio intercom; vapor concentrations are independently monitored and maintained.
- The subject reads for the first 35 minutes of exposure.
- In the remaining 40 minutes, he performs the automated portion of the task battery and completes his double-blind rating form.
- The experimenter independently completes his double-blind rating form.

Postexposure Activities
- Blood and urine samples are collected.
- Vital signs are measured, and self-report scales are completed.[b]
- The subject reads during a 20-minute rest break.
- The exposure room is purged of vapor.
- The postexposure battery is performed, and self-report scales are completed.
- Vital signs are measured, and a final urine sample is collected.
- The subject is paid for his participation, given a blank diet log for his next session, and escorted out of MRI.

[a] Physiological recording sensors are attached during this time in sessions involving battery A.

[b] The three experimenter-administered tasks are performed at this time in sessions involving battery B.

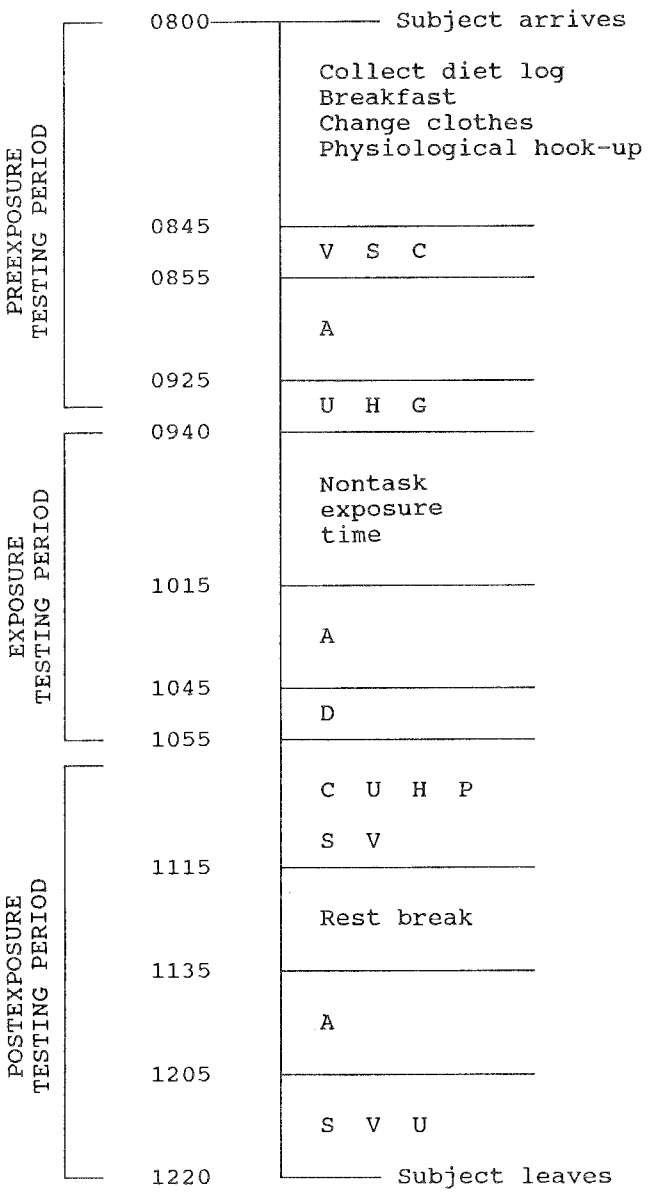


Figure 5. Time line for battery A experimental sessions. V = vital signs; S = subjective scales; C = carbon monoxide level; A = battery A; U = urine sample; H = blood sample; G = generate sham or methanol vapor; D = double-blind rating form; P = purge exposure room.

Memory is often affected by exposure to environmental toxicants, and measures of memory are included in many neurobehavioral task batteries (for example, the Baker-Letz, Microtox, and Navy Biodynamics batteries) (Gullion and Eckerman 1986). The Sternberg memory task was selected for use in this study because it can measure the speed with which the memory store can be searched, independent of decision time and motor response time (Sternberg 1975).

The ability to track visual events in space is an important component of driving. Although tracking is not included in many neurobehavioral test batteries because of the specialized equipment required, it is an important function and has been reported to be sensitive to methylene chloride (Winneke 1982), carbon monoxide (Putz et al. 1979), and ethanol (for example, Linnoila et al. 1985).

Table 3. Neurobehavioral Task Battery B

Test Sequence	Time to Perform (minutes)
Computer-Interactive Tasks (Battery B_1)	
Sternberg memory task (SMT, sets 3 and 5)	3
Visual tracking task (VTT)	1
Dual VTT/SMT task (1-minute rest break)	2
Speeded addition task (SAT)	1
Interval production task (IPT)	2
Dual IPT/SAT task (1-minute rest break)	2
Two-letter search task	2
Gamberale reaction time task	10
Experimenter-Administered Tasks (Battery B_2)	
OPTEC 2000 vision tasks	5
Hand steadiness task	2
Critical flicker fusion task	3

Mental arithmetic is the most common measure of information processing, and is included in the Walter Reed Performance Assessment (Thorne et al. 1985) and Navy Biodynamics (Irons and Rose 1985) batteries. An externally paced, speeded addition task modeled after that of Williams and Lubin (1967) was selected for the study reported here because of its sensitivity to sleep loss. Similar tasks have been used in the study of a variety of solvents (Dick and Johnson 1986).

The ability to estimate time intervals accurately is an important function in everyday life. The interval production task (Michon 1966) is a sensitive and sophisticated method for measuring this function.

A letter search task is another test in the WHO battery. The two-letter search task used in the present study is a component of the Walter Reed Performance Assessment Battery (Thorne et al. 1985). The task measures the ability to search quickly and accurately for relevant information.

The Gamberale reaction time task provides a measure of simple reaction time, which is sensitive to exposure to a variety of central nervous system depressants and toxicants, including methylene chloride (Winneke et al. 1973), methyl chloroform (Gamberale and Hultengren 1973), toluene (Gamberale and Hultengren 1972), xylene (Seppalainen et

al. 1981), acetone (Matsushita et al. 1979), alcohol (Perrine 1976; Dinges and Kribbs 1990), and carbon monoxide (Rummo and Sarlanis 1974). The measure is also included in three commonly used neurobehavioral test batteries: WHO, Baker-Letz, and Microtox (Gullion and Eckerman 1986).

OPTEC 2000 vision tester tasks (Stereo Optical Co., Chicago, IL) were included because of the well-known effects of large doses of methanol on visual function. The OPTEC provides a fast, inexpensive method for the evaluation of a wide range of visual functions.

Fine motor control is included in most neurobehavioral test batteries, and hand steadiness is often the measure of choice. In one of the earliest neurobehavioral toxicology studies, Vernon and Ferguson (1969) reported that trichloroethylene at 1,000 ppm impairs hand steadiness. Pyridostigmine (Graham et al. 1984), however, improves hand steadiness.

The critical flicker fusion task measures the temporal acuity of the visual system. It has been shown to be sensitive to methylene chloride (Winneke et al. 1973) and to trichloroethylene (Vernon and Ferguson 1969), and has been used in many other studies of solvents and central nervous system depressants (Dick and Johnson 1986).

Dual tasks are used to increase the workload experienced by the subject and to decrease reserve performance capacity. They are often included in laboratory neurobehavioral studies. Two dual tasks were used in battery B: The visual tracking task was performed together with the Sternberg memory task; and the interval production task, with the speeded addition task. Dual tasks that require the sharing of attention have proved valuable in studies of the neurobehavioral effects of carbon monoxide (Mihevic et al. 1983) and of ethanol (Smith et al. 1990).

Administration of battery B began with the Sternberg memory task (Sternberg 1975). For each trial, the subject first memorized a set of three numbers presented over headphones by a Cognitronics voice generator. A random series of 20 single numbers was then presented. The subject decided whether or not each number was one of the set of numbers memorized, and then pressed a "yes" or "no" button on his response device. Next, the subject memorized a set of five numbers, and the process was repeated. Data included reaction time for each response category, error and miss rates, and the slope and intercept of reaction time on set size.

The visual tracking task has been described in detail elsewhere (Graham et al. 1985). It measures the ability to coordinate visual and psychomotor function in tracking a continuously moving target. The object of the task was to manipulate a joystick to keep a visual target (2.5 mm) inside a moving path that was approximately 2 cm wide. Path movement was controlled by software, using a combination of three sine waves, which made the movement of the path appear both random and erratic. Root mean-squared error (MSE) served as the measure of performance.

Subjects were required to perform the visual tracking task and the Sternberg memory task both separately and together as a dual task. During the dual task, the tracking task was defined as the primary task. Subjects were told: "Remember, the tracking task is primary. Keep attending to that task. The memory task is secondary. Shift your attention in and out of that task, but try to be as quick and accurate as you can." The tracking and memory dual task was performed at two levels of secondary task difficulty (set size 3 and set size 5).

An externally paced speeded addition task, modeled after that of Williams and Lubin (1967), was used as one measure of information processing. At each trial, eight tape-recorded addition problems were presented over a loudspeaker. Each problem consisted of two two-digit numbers (for example, 57 and 36). The subject had seven seconds to add the numbers together and say the answer. Performance measures were the number of correct additions and the number of missed response intervals.

The interval production task was used to measure variability in the internal sense of time (Michon 1966). The subject first depressed a microswitch in time with a series of tones presented at 500-msec intervals over the headphones. After 20 seconds, the tones ceased, and the subject attempted to maintain the same response rate for another 100 responses. The interval production task score, based on the mean difference between successive responses, provided the performance measure.

Subjects were required to perform the interval production task and the speeded addition task simultaneously. The interval production task was defined as the primary task. Subjects were told: "Remember that the tapping task is the primary task. Put most of your attention on the tapping task, and try to keep your tapping rate as constant as you can. The addition task is secondary. Let your attention shift in and out of that task, but try to answer as quickly and accurately as you can."

To evaluate the ability to search quickly and accurately for relevant information, the two-letter search task (Thorne et al. 1985) was used. Two target letters were presented at the top of the computer screen, along with a string of 20 letters in the middle of the screen. The subject determined as quickly as possible whether or not both target letters were present in the string. If both were present, he was to press the "yes" button on his response device; if not, he pressed the "no" button. Both the search string and the target letters

changed on each of the 24 trials. Reaction time and accuracy were measured.

The Gamberale reaction time task (Iregren et al. 1986) was used to measure sustained attention. Auditory stimuli were presented at the rate of 16 per minute for 10 minutes. Within each minute, the interstimulus interval varied randomly. The subject's task was to press a microswitch as quickly as possible after each stimulus. Performance was evaluated in terms of accuracy and mean reaction time for each minute.

Standardized measures of visual function were obtained using the OPTEC 2000 Vision Tester. This device is widely used for rapid visual screening. Specific functions tested included depth and color perception, peripheral vision, binocular acuity (far and near), and vertical and lateral phoria (far and near).

A test apparatus was constructed to evaluate the ability to make fine, highly controlled adjustments of the musculature under conditions that amplify the effects of muscle tremor (hand steadiness task). The subject's task was to hold a metal stylus for 10 seconds in each of six holes of progressively smaller diameter drilled in a metal plate. Electronic logic connected to an automatic timer was used to record the cumulative number of contact seconds between the stylus and the metal plate surrounding each hole.

To measure the temporal acuity of the visual system, the critical flicker fusion frequency threshold was determined. A Grass photostimulator (Model PS22) served as the stimulus. When the stimulator flashes at a low rate, it is perceived as a flickering light. As flash rate increases, a point is reached at which the visual system is no longer capable of resolving each discrete light flash, and the light is perceived as a fused, steady light. This point is called the critical flicker fusion threshold. Three ascending and three descending trials were administered, and the mean and SD over the six determinations were calculated.

Table 2 summarizes the experimental procedures, and Figure 6 shows the time line for battery B experimental sessions.

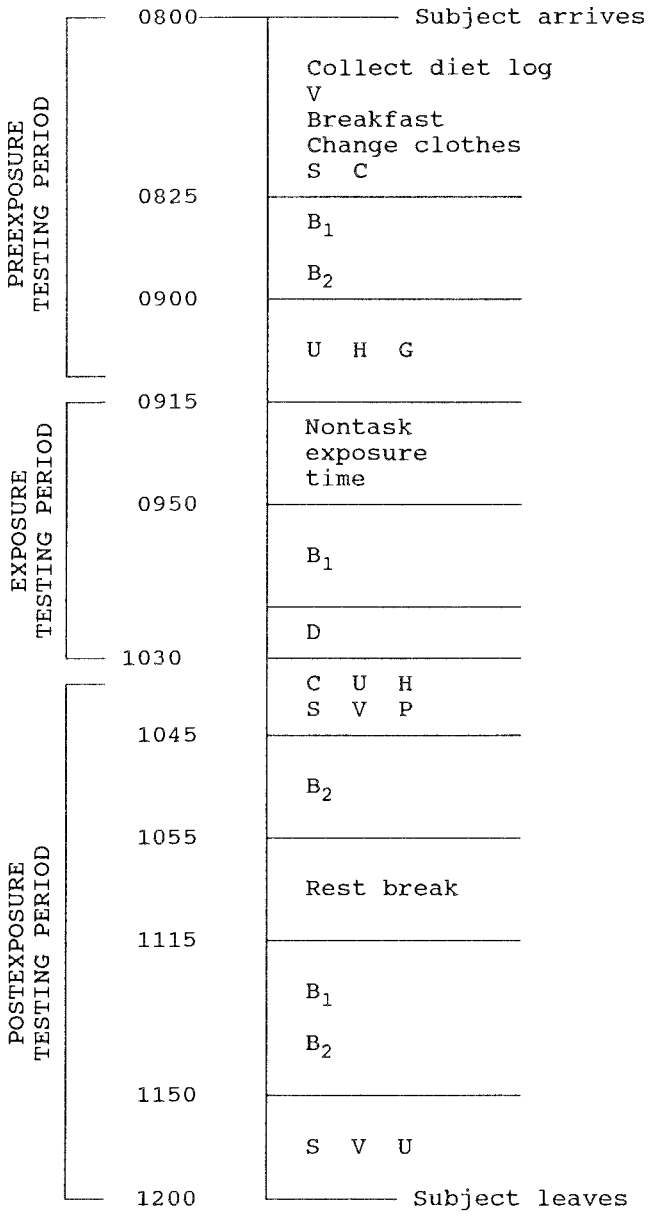


Figure 6. Time line for battery B experimental sessions. V = vital signs; S = subjective scales; C = carbon monoxide level; B_1 = computer-interactive portion of battery B; B_2 = experimenter-administered portion of battery B; U = urine sample; H = blood sample; G = generate sham or methanol vapor; D = double-blind rating form; P = purge exposure room.

QUANTIFICATION OF METHANOL AND FORMATE LEVELS

Methanol

Methanol was quantified by analyzing the headspace content of heated body fluid samples using a gas chromatograph. The method of Sedivec and colleagues (1981) for sample preparation was modified slightly to evaluate different chromatographic columns that had been reported as acceptable for the analysis of volatile chemicals, including methanol. Sample preparation and treatment were then further modified to use an automated headspace sampler rather than the manual injection method used in column evaluation. Table 4 lists the parameters used, and Figures 7 and 8 show sample chromatograms for blood and urine. A Nelson 4400 data system (P.E. Nelson, Cupertino, CA) was used to measure electronically the chromatographic peaks for methanol and for acetonitrile, the internal stan-

Table 4. Parameters Used for Analysis of Methanol Concentration in Blood and Urine

Instrument: Varian Vista 6000 (Varian Associates, Sugarland, TX) gas chromatograph
Injector: Hewlett Packard (Arondale, PA) model 19295A headspace analyzer
Injection volume: 1 mL
Injector bath temperature: 95°C
Injector valve loop temperature: 100°C
Column: Porapak QS (Waters Associates Inc., Milford, MA) 100/120 mesh, 1.8 m by 2 mm internal diameter, glass
Column temperature: 100°C, isothermal
Detector: flame ionization
Detector temperature: 200°C
Carrier gas: nitrogen
Carrier flow: 30 mL/minute (25 via injector, 5 from the gas chromatograph)
Airflow: 300 mL/minute
Hydrogen flow: 30 mL/minute

dard. Blood was used as the matrix for blood analysis standards, and urine was used as the matrix for urine samples.

To evaluate the method, urine and blood samples were spiked with methanol at 0.4, 0.8, 2.0, and 4.0 mg/L. Single injections of at least triplicate samples were made at each concentration for both urine and blood. Data from the standards in the method performance evaluation were used to calculate the relative SD. Across the entire range of concentrations, the relative SD ranged from 2 percent for 4.0 mg/L to 25 percent for 0.4 mg/L. Mean absolute error in urine was 0.1 mg/L, and in blood, 0.4 mg/L. Linearity, expressed as the correlation coefficient of the matrix standard curve, was 0.997 in urine and 0.974 in blood. The signal-to-noise ratio was 4.9. The minimum quantifiable level was defined as 10 times the SD of the lowest standard for the standard curve determined for each batch of samples analyzed.

After evaluating the analytical method, stability studies were undertaken. An aliquot of each sample was analyzed; the remaining aliquots were stored in sealed vials. Methanol in urine was stable for 60 days when the sample was stored at −20°C in a nondefrosting freezer, and there was no evidence that vaporization occurred. Urine samples were, therefore, analyzed in relatively large batches. However, the analytical data indicated that methanol was not stable in blood samples when stored for more than seven days. Blood samples were, therefore, stored at 5°C for no more than seven days.

Formate

Formate in plasma was quantified using a modification of the enzymatic method of Buttery and Chamberlain (1988).

```
FILE NUMBER :  8
Raw data file was read from KALMET109:,700,0
Method file was read from /RC/KALMEOHm:REMOTE

================================================================================
   Sample Name METHANOL

   Date: 14 Nov 1989  16:32   Method:                Operator: KAL
   Interface: 702     Cycle#:109     Channel#: A          Vial#: -1
================================================================================
Instrumental Parameters
-----------------------
Instrument: VARIAN VISTA 6000
Column: PORAPAK  QS-100/120 MESH          Column Length: 1.8M Meters
Start Temp-Time (deg-min): 100             Ramp Hold (deg-min): 15
Program Rate (deg/min): 0      End Time-Temp (deg-min): 100
Prog Slope (N or Linear):          Inj Port Temp: 150
Flowrate/Gas: 5/N2             Split Ratio: NO
Det 1-Type & Temp: FID/200                 Det 2-Type & Temp:
Notes: 26 ML/MIN FROM AUTOSAMPLER

================================================================================
                        *** AREA PERCENT REPORT ***

Data From Sample METHANOL              Collected on 14 Nov 1989  16:32
Delay Time :   0.00 min                 Run End :   15.00 min

Pk   Time       Area      Area  B   Ht     Normalized   Ar/Ht   A/D
No.  [min]    [uV-sec]     %   L  [uV]   to Max Peak   [sec]  Range
-------------------------------------------------------------------
  1   1.851    105810   3.8700 2   8028      11.162     13.2   Normal
  2   2.389    684920  25.0508 2  36356      72.251     18.8   Normal
  3   3.120    947967  34.6716 2  62626     100.000     15.1   Normal
  4   4.979    515020  18.8367 1  22526      54.329     22.9   Normal
  5   7.659    480410  17.5709 1  13987      50.678     34.3   Normal

Total Area =    2734127 uV-sec   Area Reject =        50 uV-sec
Sampling Rate =     1.00 pts/sec    Bunch Factor = 4 pts
Noise Threshold = 34.3 uV      Area Threshold = 170 uV-sec

**********  INTERNAL STANDARD REPORT  **************************
Data From Sample METHANOL              Collected on 14 Nov 1989  16:32
Delay Time :   0.00                     Run Time :   15.00
Area Reject =     50 uV-sec   Sampling Rate =   1.00 pts/sec
Bunch Factor = 4 pts
Noise Threshold = 34.3 uV      Area Threshold = 170 uV-sec
Sample Amount = 1 USER UNITS   Injection Vol = 1
Dilution Factor = 1       Multiplier Amount =     1.0000

Peak  Ret  Peak              Concentration as   Raw      Area     Cal   Peak     Ref  Int Std  Relative    Response
Num   Time  Name                 USER UNITS     Area     Ratio   Range  Type     Peak  Peak   Ret Time     Ratio
-------------------------------------------------------------------------------------------------------------
 2   2.39 METHANOL              1.42570      684920   1.4256989 Over    2        2     5      1          1.0000
 5   7.66 ACETONITRILE          0.00000      480410   1.0000000  -       1        2     IS     3.2057     1.0000
-------------------------------------------------------------------------------------------------------------
Total Amount =          1.42570
  NOTE: The Internal Standard Is Not Counted In The Total Amount

 Start time=    0.00              Stop time=   15.00 minutes
Lowest Value =     7.270 mV  Highest Value =   137.900 mV Scale factor =   10
```

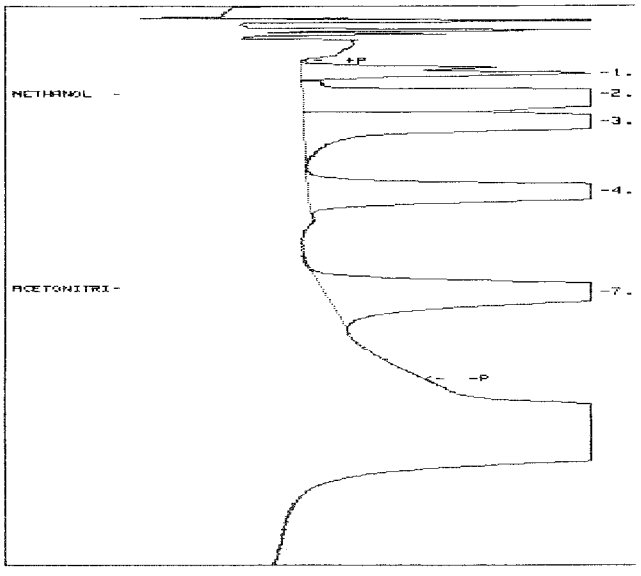
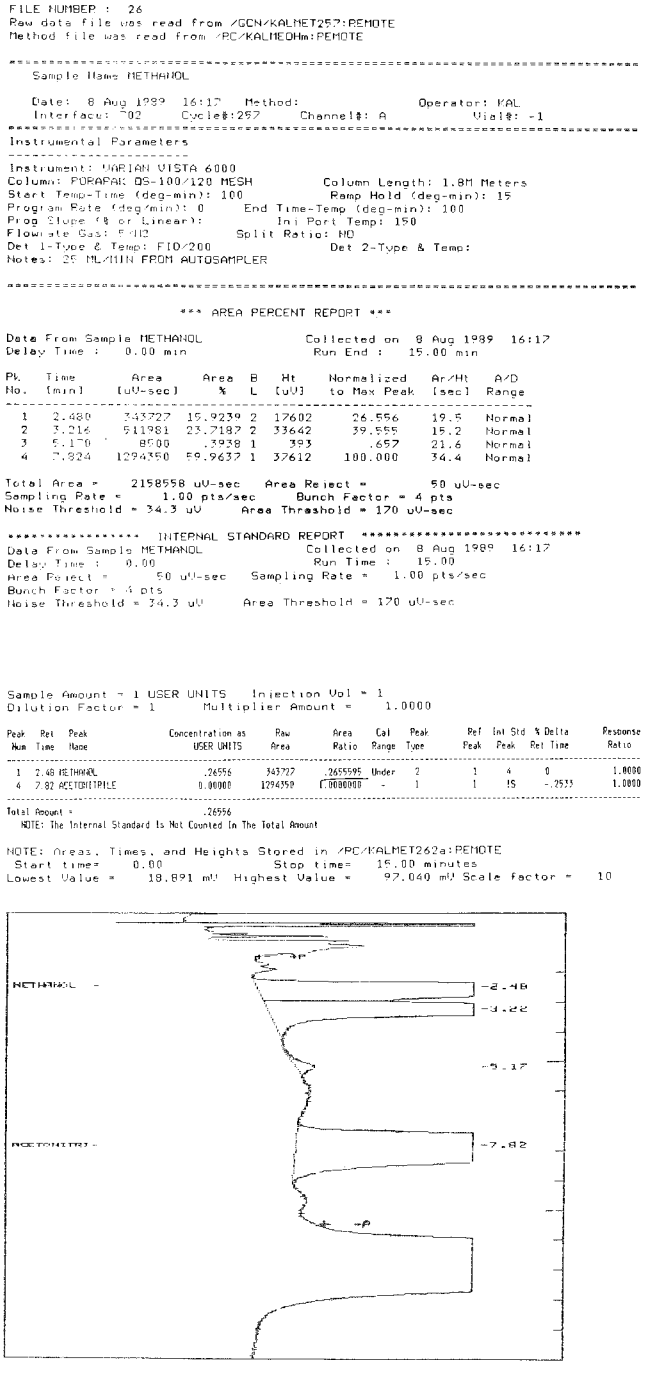


Figure 7. Sample chromatogram for blood methanol quantification.



```
FILE NUMBER :  26
Raw data file was read from /GCN/KALMET257:REMOTE
Method file was read from /PC/KALMEOHm:REMOTE

   Sample Name METHANOL

   Date:  8 Aug 1989  16:17   Method:                Operator: KAL
   Interface: 702     Cycle#:257     Channel#: A          Vial#: -1

Instrumental Parameters
Instrument: VARIAN VISTA 6000
Column: PORAPAK QS-100/120 MESH          Column Length: 1.8M Meters
Start Temp-Time (deg-min): 100           Ramp Hold (deg-min): 15
Program Rate (deg/min): 0       End Time-Temp (deg-min): 100
Prog Slope (# or Linear):          Inj Port Temp: 150
Flowrate Gas: N2                Split Ratio: NO
Det 1-Type & Temp: FID/200              Det 2-Type & Temp:
Notes: 25 ML/MIN FROM AUTOSAMPLER

                    *** AREA PERCENT REPORT ***

Data From Sample METHANOL              Collected on  8 Aug 1989  16:17
Delay Time :   0.00 min                 Run End :   15.00 min

Pk  Time       Area      Area  B   Ht    Normalized   Ar/Ht   A/D
No. [min]    [uV-sec]     %   L  [uV]   to Max Peak  [sec]  Range
  1   2.480    343727  15.9239 2  17602     26.556     19.5   Normal
  2   3.216    511981  23.7187 2  33642     39.555     15.2   Normal
  3   5.170      8500   .3938 1    393       .657     21.6   Normal
  4   7.824   1294350  59.9637 1  32612    100.000     34.4   Normal

Total Area =    2158558 uV-sec   Area Reject =        50 uV-sec
Sampling Rate =    1.00 pts/sec     Bunch Factor = 4 pts
Noise Threshold = 34.3 uV      Area Threshold = 170 uV-sec

*****************  INTERNAL STANDARD REPORT  ***************************
Data From Sample METHANOL              Collected on  8 Aug 1989  16:17
Delay Time :   0.00                     Run Time :   15.00
Area Reject =      50 uV-sec    Sampling Rate =   1.00 pts/sec
Bunch Factor = 4 pts
Noise Threshold = 34.3 uV      Area Threshold = 170 uV-sec

Sample Amount = 1 USER UNITS   Injection Vol = 1
Dilution Factor = 1       Multiplier Amount =    1.0000

Peak  Ret  Peak          Concentration as   Raw       Area   Cal   Peak      Ref  Int Std  % Delta      Response
Num   Time Name             USER UNITS      Area      Ratio  Range Type      Peak  Peak   Ret Time      Ratio
  1  2.48 METHANOL          .26556        343727   .2655595 Under  2         1     4      0           1.0000
  4  7.82 ACETONITRILE      0.00000      1294359   .0000000   -     1         1     15     -.2533      1.0000

Total Amount =            .26556
  NOTE: The Internal Standard Is Not Counted In The Total Amount

NOTE: Areas, Times, and Heights Stored in /PC/KALMET262a:REMOTE
 Start time=    0.00              Stop time=   15.00 minutes
Lowest Value =   18.891 mV  Highest Value =   97.040 mV Scale Factor =   10
```


Figure 8. Sample chromatogram for urinary methanol quantification.

The following modifications were made to increase the accuracy and sensitivity of the method: (1) plasma from the subject, rather than from a second source, was used to obtain standard addition data; (2) the volumes of plasma and reagents were increased, and the volume of the diluent decreased; and (3) the reagents were added in two separate steps, followed by separate incubations.

Table 5. Reagents Used for Quantification of Formate in Plasma

Buffer: 0.05 M potassium phosphate, monobasic, in deionized water; pH adjusted to 7.4.
Buffered NAD solution: 200 mg of NAD (Sigma Chemical Co., St. Louis, MO, N-1511) in 20 mL of buffer.
Enzyme solution: 30 mg of formate dehydrogenase (Boehringer Mannheim, Minneapolis, MN) in 4 ml of buffer.
Spiking solution: 0.4 mM sodium formate in deionized water.
Color reagent: 5 mg of phenazine methosulfate (Sigma P-9625) in a clarified, decanted solution of 80 mg of *p*-iodonitrotetrazolium violet (Sigma I-8377) in 20 ml of deionized water.
Diluent: 0.1 N hydrochloric acid.

Blood samples were drawn in heparinized Vacutainers and placed in an ice and salt bath. Plasma was separated by centrifugation, and the plasma was kept in an ice and salt mixture during the analysis. Each plasma sample was analyzed in triplicate and accompanied by triplicate method blanks (reagents other than enzyme in plasma) and spikes. Reagents were added to one test tube at a time, and reagent additions were carefully timed. Table 5 lists the reagents used.

Buffered nicotinamide adenine dinucleotide (NAD) solution (200 µL) was added to each test tube, followed by the addition of 125 µL of the subject's plasma. Spiking solution (125 µL) was added to the spike test tubes, and an equivalent volume of deionized water was added to the sample and method blank tubes. Enzyme solution (40 µL) was added to the sample and spike test tubes, and an equivalent volume of deionized water was added to the method blank tubes. The solutions were then incubated in a water bath protected from light for 30 minutes at 37°C. Color reagent (200 µL) was added to each tube, followed by an additional 10-minute incubation. Diluent (3 mL) was added and the absorbance of the solutions measured at 510 nm against a water blank. Formate concentrations in millimoles per liter were calculated by

$$[(SA - MA)/(SP - SA)] \times SPC,$$

where *SA* is sample absorbance, *MA* is method blank absorbance, *SP* is spike absorbance, and *SPC* is spike concentration.

Method evaluation indicated a range of relative SDs from 0.85 percent (0.1 mmol/L) to 2.61 percent (0.4 mmol/L).

Linearity was 0.997, and percentage of relative error was equal to or less than 15.1 percent (0.1 mmol/L).

Spectrophotometer absorbance values for plasma spiked with 0.04 to 1.0 mmol/L of plasma formate were 0.528 to 1.213. The slope of the standard curve of quantification from 0.04 to 1.0 mmol/L was 0.7133. Formate concentrations were calculated using the mean of three analyses of each of the following: blank, sample, and spiked sample. Day-to-day variations, obtained by adding the relative SDs (percent) of the mean values used in the final calculation formula, ranged from 2.0 to 30.0 percent over the entire study period. However, the typical sum of the relative SDs (percent) for the mean values on any given day was approximately 10 percent. Using the test subjects' own plasma as a blank for the enzymatic quantification method precludes any problems with variability from one test individual to another.

The low plasma formate values are a result of the well-controlled diets of the test subjects in the 12 hours before each experimental session. Because of the low formate values, we optimized the enzymatic procedure described by Buttery and Chamberlain (1988), which has been used for quantifying endogenous and abnormal formate levels. The enzymatic method was not validated by an alternative method because it is known to be one of the most sensitive and cost-effective formate assays. Recent advances in ion chromatography and bioluminescent assays would probably provide even more sensitive methods. However, additional development time would have been required to quantify formate in serum or plasma samples using these techniques.

QUALITY ASSURANCE PROCEDURES

Exposure

To provide valid data on exposure levels and test conditions, high levels of quality assurance and quality control were maintained and redundant analyses were conducted. Test room methanol concentration was measured each minute with a MIRAN® infrared analyzer. Automated gas chromatographic and flame ionization detector analysis provided a secondary measure of test room methanol concentration, and was conducted every six minutes. The sampling rate was high (30 L/minute) to provide a rapid instrumental response and to minimize any potential losses in the sampling system. The sampling port was located 36 inches from the floor and 36 inches from the chair in which the subject sat during exposure.

Linearity of both the MIRAN® and gas chromatographic systems was demonstrated by analysis of specially prepared samples of methanol at 200, 250, and 300 mg/m^3. A one-point calibration was performed at the beginning and a check standard was performed at the end of each test day using specially prepared methanol standards (250 mg/m^3 ± 2 percent). The instruments never failed the objective of ± 10 percent. Precision was demonstrated at the beginning and end of data collection by four consecutive analyses of 250 mg/m^3 standards under the operating conditions used during exposure monitoring. Variability of the measures was less than 1 percent. Precision data were also obtained using duplicate runs performed during each exposure experiment.

A performance sample was analyzed each week to demonstrate the accuracy of the analysis. Performance samples were provided by another laboratory within MRI at a concentration within the working range (200 to 300 mg/m^3) but unknown to the analyst. The accuracy obtained was better than the objective of 75 to 125 percent of true value (mean accuracy 98.8 percent, SD 4.5 percent, range 88 to 105 percent).

To determine whether or not the mixing of methanol with room air in the exposure room was adequate, methanol vapor was generated and monitored using the MIRAN® in the normal mode. The sampling inlet of a second MIRAN® was placed in 11 different positions in the room, and the difference between the readings on the two MIRAN® monitors was calculated as a percentage of the first MIRAN®. During the monitoring test, the chamber was operated in the normal manner. One "subject" was in the chamber, and remained seated in the usual position for subjects, except for the brief period when the position of the movable inlet was changed. The movable sampling inlet was placed in all four corners of the room, 1 foot from the floor and 2 feet from the ceiling. Three measurements were taken where the subject's chair was located; one at lap level, one at mouth level, and one above the subject's head. The difference between the two MIRAN® measurements ranged from 0.0 to 2.4 percent, with an average of 0.7 percent.

Cognitive and Performance Data

Instruments used for obtaining behavioral and cognitive data were calibrated weekly to ensure that all equipment was operating according to the manufacturers' specifications. Computer programs used for task presentation and data collection were documented and verified. All data were marked with a unique code number that indicated the subject identification number, the experimental session, the testing period within the session, and the specific task performed.

Physiological Data

Instruments used to collect physiological data were fully calibrated at the beginning and end of the data collection

period. Direct current (DC) absolute voltage values for all Beckman recorder channels were calibrated with a DC microvolt instrument with National Bureau of Standards traceability. Frequency response of all recording channels was evaluated against the manufacturer's specification (Beckman R-611/R-612 Dynograph Recorder Service Manual) using laboratory-grade testing equipment. Noise checks were performed on both the recorder and the A/D converter to ensure that the manufacturer's specifications were met. In addition, all EEG recording channels were dynamically calibrated each week using an internal program; values were stored and used in the calculation of values for all EEG measures. Before the beginning of each experimental session, the electrocardiogram channel was calibrated by the experimenter. A paper record of the calibration was produced. The same data coding system described above for neurobehavioral test data was used.

Methanol and Formate Levels

Standard operating procedures were followed for the operation, calibration, and maintenance of all equipment utilized for the analysis of methanol and formate levels. For methanol, a standard curve with a minimum of triplicate matrix standards at each of five concentration levels was prepared for each batch of samples analyzed. When batches were large enough to require more than one day for completion, three independently prepared check standards were analyzed at the beginning of each day. On one occasion, the check standards disagreed with the standard curve, and a new standard curve was constructed.

For formate, the standard addition method was used to determine concentrations. Plasma samples were spiked with known amounts of sodium formate, and the value of the spiked sample was compared to that of the unspiked sample. All samples were analyzed in triplicate, and method blanks were analyzed with each sample.

All samples were uniquely identified with a number indicating the subject, the experimental session, and the testing period within the session. Data were reviewed by peers and a supervisor before being submitted to statistical analysis.

Data Management

Data were transferred to the data manager either on computer disks or on hand-recorded data sheets. The data manager logged in and reviewed all data for completeness and kept a list of procedural deviations, problems encountered, and comments made by the experimenters. All hand-recorded data were entered into a computer data-base manager file. Entries were checked by a second staff member. All physiological data that required data reduction were first processed by a member of the project team and then checked by a senior staff member with extensive experience with the variable of interest. Computer files were then prepared for analysis and checked by the data manager.

STATISTICAL METHODS AND DATA ANALYSIS

ELIGIBILITY OF EXPERIMENTAL SUBJECTS

To be eligible for participation in the study, subjects had to meet the following criteria: be a male 21 to 35 years of age; be a nonsmoker for at least 12 months; have no history of chronic disease or disability; have had no recent acute illness requiring more than three days of bed rest; have normal hearing and normal (corrected) vision; be willing to undergo a medical examination; be willing to eliminate alcoholic beverages and food and drink containing aspartame from the diet for 24 hours before each experimental session; and be willing to drastically restrict food intake for 12 hours before each experimental session. Potential subjects who met these initial criteria were sent to the project physician, who obtained a detailed medical history and performed a physical examination that included an ECG and extensive blood chemistry analysis. Only those volunteers who were approved by the project physician were entered into the experiment.

METHODS USED FOR BLINDING OF OPERATIONS

Subjects were randomly assigned to testing orders by the Principal Investigator before the beginning of subject recruitment. The methanol or sham exposure condition assigned for each experimental session was written on a piece of paper and sealed in an envelope labeled with the subject number and experimental session number. The envelope was usually given to the exposure facility manager on the afternoon before each experimental session; if the Principal Investigator was to be away, the sealed envelope was given to the facility manager before her departure. The master subject list was also sealed in an envelope and was kept in a locked file accessible only to the Principal Investigator.

To reduce the probability that either the subject or the experimenter would be aware of whether or not methanol or sham vapors were being presented in any given experimental session, all procedures for the two types of sessions were identical, except that on sham exposure days filtered air was entered into the methanol generation system rather than methanol. Performing an experiment under double-blind conditions does not, of course, ensure that subjects or experimenters will not be able to detect the conditions. For

this reason, both subjects and the experimenter who collected neurobehavioral data completed a questionnaire after every exposure. They judged whether methanol was present or not, rated the degree of confidence they felt in the judgment, and gave their reasons for the judgment. Subjects made the judgments for two time periods: when they first entered the test room for the exposure, and just before they left the test room.

NUMBER OF OBSERVATIONS AND MISSING DATA

All 12 subjects completed the experiment. Occasionally, however, as a result of equipment problems or experimenter error, a data point was missed. This occurred for 15 of the 4,152 planned data points (0.36 percent).

Missing data points have important consequences for data that are to be submitted to repeated measures analysis of variance, because data are required for every cell in the analysis. There are three accepted methods for addressing the problem of missing data points. The subject can be dropped from the analysis of the variable for which some data are missing. This is the most conservative approach, but it reduces the number of degrees of freedom available and is typically used only when no other viable method is available. This approach was used for 6 of the 15 missing data points. Another method is to substitute data from the same subject during the same testing period and exposure condition for the missing data point. For example, one blood sample for methanol analysis was improperly stored. Because the urinary methanol values for the same subject for the same testing periods of the two exposure days were not different, it was possible to substitute the blood methanol value from the other exposure day. This approach was used for four data points. A third method is to estimate the data point from the mean of other data points from the same subject. For example, a preexposure formate sample was lost because of a processing error. The mean of the other three preexposure samples for the same subject was used to estimate the missing data point. This approach was used five times.

STATISTICAL PROCEDURES AND ANALYSIS PROGRAMS

Data were entered into computer files by subject and experimental session number. All values were verified, and decisions were made about the treatment of missing values, before the double-blind code was broken. The subject assignment codes were then entered into the computer, and Statistical Analysis Systems software was used to sort all variables by exposure category and order. A correlation matrix of all variables other than the experimental control variables was computed (Statistical Analysis Systems PROC CORR) on the preexposure testing period of the first experimental session, and cluster analysis was performed using BMDP1M software. Cluster analysis, together with examination of the correlation matrix, was used to group variables together empirically for multivariate analysis of variance. To the extent possible, all variable groupings that were correlated at $p \leq 0.01$ were entered into a single multivariate analysis. This was not possible, however, for the event-related potential measures, as it would have required too many variables in the multivariate run. The strategy for analysis of event-related potential measures is described in detail in the Physiological Measures section under Results. Variables that did not correlate significantly with other variables were submitted to univariate analysis. Because specific hypotheses were being tested about methanol and formate levels, univariate analysis was used for these variables. These procedures reduced the initial statistical tests required from 103 to 30.

The Greenhouse and Geisser (1959) technique was used to correct F values for inflated degrees of freedom due to repeated measures. Unless otherwise specified, all probability levels reported for univariate or multivariate analysis of variance are the corrected values. Degrees of freedom reported are uncorrected to indicate the actual number of observations in the F values.

Double-blind ratings were analyzed by hand using Cochran's Q and chi-squared test with Yates correction. Analysis of variance (BMDP4V software for multivariate and univariate analysis of variance) was used for all other variables. The program automatically provides univariate analyses for all of the variables in a multivariate analysis. Univariate analyses were not interpreted except when there was a significant multivariate effect ($p < 0.05$) or trend ($p < 0.10$), or when the dependent variable was uncorrelated with other dependent variables. Significant univariate interactions were further examined using simple effects tests.

Finally, the relationship between changes in variables that showed exposure effects and changes in blood levels of methanol as a function of exposure was examined using the New Regression program of the Statistical Package for the Social Sciences (SSPS Inc., Chicago, IL).

The design of the experiment had specific implications for the interpretation of results. A significant exposure-by-testing-period interaction (subjects exposed to sham and methanol are equivalent before the exposure, but different during or after, or both during and after, the exposure) would provide the clearest indication that methanol affected the variable. When practice or fatigue effects were present, significant exposure-by-order-of-exposure effects should be

observed; examination of the means and simple effects analysis can be used to determine whether or not the effect is greater than could be explained by practice or fatigue.

RESULTS

Many measures were obtained in the present study. The probability of finding a significant effect simply by chance increases with the number of statistical tests performed. Procedures to reduce the number of statistical tests required, and to decrease the probability of rejecting the null hypothesis when it should have been accepted, were, therefore, followed. The total number of analyses required was reduced empirically by grouping correlated variables together for multivariate analysis of variance. Significant multivariate effects were followed up by univariate analyses. Univariate analysis of variance was performed on uncorrelated variables. However, to provide other investigators with data to guide future research, the univariate exposure-by-testing-period interactions for all variables are presented in Appendix C. Some univariate interactions appeared to be significant, but were not interpreted because no significant multivariate effect was found. Footnotes in Appendix C identify such variables.

EXPERIMENTAL CONTROL VARIABLES

Test Room Methanol Concentrations

Data on methanol concentrations were taken from a logging computer that recorded MIRAN® measurements once each minute and gas chromatographic values once every six minutes throughout the experimental session. For each methanol exposure, MIRAN® values for each minute during the exposure were averaged and the SD was obtained. The data are presented in Table 6. Over all subjects and experimental sessions, mean methanol concentration measured with the MIRAN® was 249 mg/m^3, with a SD of 7 mg/m^3. It is clear from Table 6 that methanol concentrations clearly met the objective of variability less than or equal to 10 percent.

Environmental Control Variables

Environmental conditions were monitored to determine whether or not any differences between sham and methanol exposure conditions were large enough to affect the neurobehavioral test results. The results are summarized in Table 7. Temperature, relative humidity, and barometric pressure were sampled once per minute during the 75-minute sham and methanol exposures. For these variables, a point 15 minutes after the beginning of the test battery during the exposure was selected for analysis of variance. No significant differences in temperature as a function of order of exposure, exposure condition, or day were found. Relative humidity was not different on methanol and sham exposure days, but was slightly higher on the second methanol exposure day than the first (F = 8.21, degrees of freedom [df] 1,10, MSE = 17.90, p = 0.02). Barometric pressure was higher on sham exposure days than on methanol exposure days (F = 8.17, df 1,10, MSE = 0.026, p = 0.02), but this was true only for subjects exposed in the order methanol-sham (F = 10.13, df 1,10, MSE = 0.026, p = 0.01). However, the absolute difference for this group was quite small (29.24 versus 28.96).

Test room carbon monoxide levels were measured before and after exposure. Analysis of variance revealed that, as expected from local traffic patterns, levels were slightly higher at the beginning of the experimental session than at the end (3.13 versus 2.17 ppm; F = 9.16, df 1,7, MSE = 1.73, p = 0.02). There was also a trend for this difference to be

Table 6. Mean Methanol Concentrations in the Test Room Calculated Over 75-Minute Methanol Exposures

	First Methanol Exposure		Second Methanol Exposure	
Subject	Mean	SD	Mean	SD
01	247	4	252	3
02	244	21	245	13
03	251	5	261	4
04	244	4	248	5
05	249	4	245	4
06	248	3	250	3
07	253	15	250	4
08	250	2	250	4
09	250	4	250	2
10	250	3	250	3
11	250	2	249	3
12	250	2	249	4

Table 7. Levels of Environmental Variables for Methanol and Sham Exposure Sessions

	Exposure Condition			
	Sham Exposures		Methanol Exposures	
	Mean	SD	Mean	SD
Ambient temperature (°F)	76	2.6	76	2.2
Relative humidity (percent)	55.9	12.5	56.2	10.3
Barometric pressure (in Hg)	29.19	0.19	29.06	0.28
Carbon monoxide (ppm)	2.21	1.23	3.09	2.03

greater on methanol than on sham days (F = 4.14, df 1,7, MSE = 1.71, p = 0.08). This was due to a chance relationship between the experimental schedule and an inversion in the metropolitan area that lasted for a few days. The absolute levels of carbon monoxide observed in the test room were well below the levels shown to have neurobehavioral effects (Dick and Johnson 1986). No systematic changes in levels of carbon monoxide in expired air as a function of exposure were observed.

Double-Blind Ratings

At the end of each exposure, the experimenter judged whether or not methanol or sham vapors had been presented. The first block of Table 8 summarizes the results. Judgments were accurate 69 percent of the time (χ^2 = 5.58, df 1, $p < 0.02$), and accuracy was positively related to the experimenter's confidence in his judgments. Examination of the reason for making each judgment suggested that the experimenter learned to recognize the odor of methanol. Over the first 12 judgments, he was correct only 50 percent of the time, and odor was not a cue for any judgment. Over the second 12 judgments, accuracy increased to 75 percent, and odor was a cue for 6 of the 9 correct ratings. Over the final 24 judgments, accuracy remained at 75 percent, and 15 of the 18 judgments were based on odor or the lack of odor.

Despite significant accuracy, the experimenter had relatively low confidence in his judgments. Confidence was rated on a scale from one (not at all confident) to five (totally confident). Of the 48 judgments, 27 had a confidence rating of one, 16 a rating of two, and 5 a rating of three. Discussion with the experimenter after analysis of the double-blind ratings indicated that his judgments were based on the initial few seconds, when he was escorting the subject into the test room. The odor was described as "very faint, like the smell you get when you first open a plastic container." If he did not detect it within the first five seconds, he was unable to detect it subsequently.

At the end of the exposure, each subject also judged whether or not methanol had been present (1) when he first entered the test room and (2) just before he left the test room. Results are summarized in the second block of Table 8. Cochran's Q was calculated to determine whether or not the subjects' double-blind judgments differed as a function of methanol versus sham exposure, experimental session number, or first versus second judgment. They did not. The chi-squared test with Yate's correction was used to determine if the subjects were able to judge the presence of methanol with better-than-chance accuracy. All eight judgments from each subject were included in the analysis; the chi-squared value might, therefore, be inflated as a result of correlations among the judgments. Overall, subjects were unable to judge the presence of methanol at better than chance levels (χ^2 = 3.53, df 1, not significant). Furthermore, when the first and second judgments were analyzed separately, neither was more accurate than chance (χ^2 = 1.35 and 1.46 respectively, df 1, not significant).

Table 8. Double-Blind Judgments

	Actual Exposure	
	Sham	Methanol
Experimenter's Judgment		
Sham	19	10
Methanol	5	14
Subjects' Judgment		
Sham	34	24
Methanol	14	24

Because the experimenter was able to judge correctly the presence of methanol at better-than-chance levels, the subjects' ratings from the experimental sessions in which the experimenter was correct were analyzed separately. Subjects were not more accurate than chance in these sessions, suggesting that they were not influenced by the experimenter's judgments. Even when only high-confidence judgments (rated four or higher) were analyzed, subjects were not more accurate than chance (χ^3 = 0.08, df 1, not significant).

These analyses indicate that the double-blind control conditions were partially effective. The experimenter learned over time to detect exposure status. However, this had no influence on the subjects' judgments, and overall, subjects were not able to detect accurately the presence of methanol.

METHANOL AND FORMATE IN BODY FLUIDS

Methanol

Exposure to methanol vapors increased the methanol concentration in both blood and urine (blood: F = 583.33, df 1,10, MSE = 0.019, p = 0.0001; urine: F = 124.43, df 2,20, MSE = 0.066, p = 0.0001). Table 9 summarizes the results. Immediately after exposure to methanol vapor at 250 mg/m^3 for 75 minutes, blood methanol concentrations tripled and urinary methanol concentrations doubled. Urinary methanol did not return to base line by the end of the experimental session. Both hypotheses with regard to methanol concentrations in body fluids were supported. A table of raw data is shown in Appendix B.

Table 9. Methanol Concentrations in Blood and Urine[a]

	Urine		Blood	
	Sham	Methanol	Sham	Methanol
Before exposure	1.022 (0.356)	0.888 (0.353)	0.604 (0.310)	0.570 (0.305)
Immediately after exposure	1.012 (0.381)	2.196 (0.576)	0.551 (0.314)	1.881 (0.468)
End of experimental session	0.889 (0.318)	2.281 (0.724)	—	—

[a] Values are means ± SD given in milligrams per liter.

Formate

As expected because of the timing of blood samples, exposure to methanol vapors had no significant effect on blood formate levels. The results are summarized in Table 10. Paired t tests between methanol and sham conditions before exposure confirmed that there was no significant difference (t = 0.56, df 11, not significant). A table of raw data is shown in Appendix B. The preexposure plasma formate values are at or a little below endogenous values reported in the literature. Buttery and Chamberlain (1988) reported plasma formate values for 30 normal individuals ranging from 0.12 to 0.28 mmol/L. Osterloh (1986) stated that endogenous serum formate concentrations have been reported from 0 to 63 mg/L, but noted an average value of approximately 4 mg/L (approximately 0.1 mmol/L) and an upper limit of approximately 12 mg/L. Endogenous formate serum concentrations as high as 1 to 2 mg/dL (10 to 20 mg/L) may occur in individuals whose diets have not been controlled.

SUBJECTIVE MEASURES

Fatigue and Workload

Fatigue and workload scales were not correlated, and univariate analysis was used. Workload ratings increased during the experimental session (F = 19.94, df 2,20, MSE = 0.48, p = 0.001). Fatigue ratings showed a trend toward the same effect (F = 3.02, df 2,20, MSE = 1.24, p = 0.097), but the increase was greater when subjects were exposed to methanol (exposure-by-testing-period interaction, F = 2.97, df 2,20, MSE = 0.31, p = 0.10).

Table 10. Formate Concentrations[a]

	Sham	Methanol
Before exposure	0.0785 (0.0258)	0.0820 (0.0243)
Immediately after exposure	0.0755 (0.0258)	0.0775 (0.0235)

[a] Values are means ± SD given in millimoles per liter.

Mood Adjective Check List

On the basis of the correlations between scales, two computed scales, each consisting of several Mood Adjective Check List factors, were derived from each administration of the check list, and these mean scores were submitted to multivariate analysis of variance. A multivariate exposure-by-testing-period interaction was observed (Wilk's lambda = 0.503, F = 3.90, df 4,38, p = 0.01), which appeared to arise from both variates.

To interpret this effect, each of the 11 scales was submitted to univariate analysis. No significant exposure effects or trends were found for the aggression, anxiety, surgency, sadness, skepticism, social affection, or egotism scales. Subjects had higher elation scores on days when they were exposed to sham vapors than on methanol exposure days (F = 6.02, df 1,10, MSE = 1.039, p = 0.03). Examination of significant three- and four-way interactions revealed that this was a spurious effect arising from two factors: individual differences, and higher scores at the beginning of the first experimental day, regardless of exposure condition.

A significant exposure-by-testing-period-by-order-of-exposure effect was found for vigor (F = 3.73, df 2,20, MSE = 1.46, p = 0.04). The effect is shown in Figure 9. When the two order groups were analyzed separately, the exposure-by-testing-period interaction approached significance for subjects exposed in the order methanol-sham (F = 4.01, df 2,10, p = 0.07), but not for those exposed in the order sham-methanol (F = 1.49, df 2,10, p = 0.28).

A trend toward an exposure-by-testing-period interaction was found for the concentration scale (F = 2.84, df 2,20, MSE = 0.668, p = 0.09). Subjects reported less concentration during the exposure when they were exposed to methanol than when they were exposed to sham vapors.

Exposure to methanol vapors was associated with a significant increase in the fatigue scale score during the exposure (exposure-by-testing-period interaction, F = 5.34, df 2,20, MSE = 1.338, p = 0.02). This finding is consistent with the results from the less sensitive USAF School of Aerospace Medicine fatigue scale. Figure 10 shows the results from both instruments.

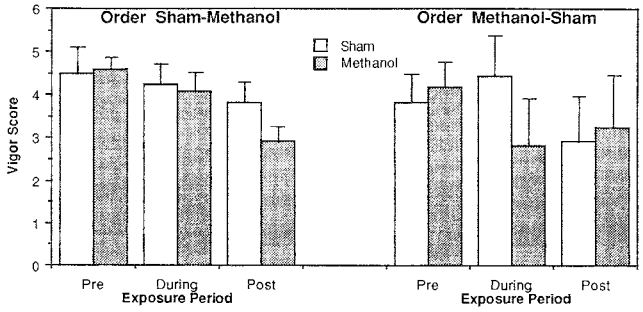


Figure 9. Vigor scores from the Mood Adjective Check List. Changes in vigor are shown as a function of exposure to methanol at 250 mg/m^3, and of order of exposure to methanol and sham vapor. Error bars for the preexposure testing period are the standard error of the mean; error bars for the exposure and postexposure testing periods are the standard error of the change from the preexposure testing period.

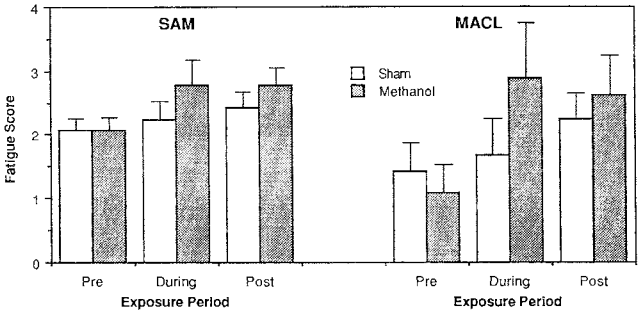


Figure 10. Fatigue scores from the USAF School of Aerospace Medicine (SAM) Scale and the Mood Adjective Check List (MACL). Increased subjective fatigue ratings are shown as a function of exposure to methanol at 250 mg/m^3. Error bars for the preexposure testing period are the standard error of the mean; error bars for the exposure and postexposure testing periods are the standard error of the change from the preexposure testing period.

Alertness Ratings

Alertness ratings were obtained in association with data on vital signs, the contingent negative variation, the two event-related potential tasks, and the Gamberale reaction time task. No multivariate effects associated with exposure were found for the alertness measures.

Health Effects Questionnaire

The Health Effects Questionnaire was administered at the beginning and end of each experimental session. Visual examination of the data revealed that subjects seldom reported any symptoms, and that the few symptoms reported showed no particular pattern. No formal statistical analysis was performed because there was so little variance in the data set.

VITAL SIGNS

No multivariate effects of exposure to methanol vapors were observed; the expected changes consistent with circadian rhythms were found.

VISUAL FUNCTION

Based on cluster analysis, data from the OPTEC Vision Tester were grouped into two sets for separate multivariate analyses. One analysis included all phoria measures, and one included measures of acuity and depth perception. Measures of color perception were not included, because there was no variance. No multivariate effect of exposure was found for either set of OPTEC measures. In addition, no significant effects of exposure were found for critical flicker fusion frequency.

PERFORMANCE TASKS

Reaction Time

Reaction time to presentation of the target stimuli in both the auditory- and visual-event-related potential tasks was determined. Multivariate analysis revealed no significant exposure effects. During the Gamberale reaction time task, which was used as a measure of sustained attention, reaction time deteriorated as expected during the task (F = 6.14, df 9,90, MSE = 0.0023, p = 0.009), but this change was not significantly affected by exposure to methanol.

Three correlated variables were obtained from the choice reaction time task. Reaction time, the SD of reaction time, and the percentage of correct responses were analyzed with multivariate analyses of variance. No significant multivariate effects were found, nor were there multivariate trends involving exposure.

The two-letter search task provided a more complex level of choice reaction time. Multivariate analyses of reaction time and of number of correct responses yielded significant interactions with exposure condition. Examination of univariate analysis revealed no significant effects for the number of correct responses. Reaction time improved through the experimental session (F = 3.96, df 2,20, MSE = 0.142, p = 0.042) and, as expected, it took longer to respond to mismatched trials than to matched trials (F = 21.19, df 1,10, MSE = 4.645, p = 0.001). These factors interacted with both exposure condition and order of exposure. The two groups with different orders of exposure were, therefore, analyzed separately. No significant exposure effects were found for either group; subjects performed better on the second day than on the first day, regardless of exposure condition. Thus, the initial significant multivariate interactions involving exposure arose from changes in performance over time.

Information Processing

The Symbol Digit substitution task measures information processing under time pressure. Subjects had longer reaction times on the first experimental day than on the second, regardless of whether methanol or sham exposure conditions were in effect. This led to a significant exposure-by-testing-period-by-order-of-exposure interaction, which was not a function of actual methanol exposure.

The Stroop color-word test measures interference with attention processes. Subjects worked more quickly on the second day than on the first (exposure-by-order-of-exposure interaction; F = 10.30, df 1,10, MSE = 33.54, p = 0.01), and were more accurate in their performance (F = 9.23, df 1,10, MSE = 33.50, p = 0.01). As expected, both number of items completed and accuracy were significantly greater for the control condition than for the interference condition. There was a trend toward an exposure-by-testing-period-by-task-condition interaction (F = 3.59, df 2,20, MSE = 12.16, p = 0.054). Further analysis of the interference condition revealed that the interaction effects could be attributed to practice; subjects performed better on the second experimental day regardless of exposure conditions.

Dual Tasks

The Sternberg memory task was performed alone, and together with the visual tracking task; tracking served as the primary task. No changes in either single or dual tracking performance as a function of exposure to methanol were found. As expected, reaction time on the memory task was slower when dual tasks were presented (F = 14.84, df 1,10, MSE = 203,232, p = 0.003), and slower when subjects were required to scan for five digits than when they scanned for only three (F = 41.99, df 1,10, MSE = 27,609, p = 0.0001). Other expected significant differences related to the task itself were also found. There was a trend for performance to be affected by exposure to methanol vapors (F = 3.57, df 2,20, MSE = 7,320, p = 0.055). The interaction is shown in Figure 11.

When exposed to sham vapors, subjects showed small decrements in performance over the course of the experimental sessions. When they were exposed to methanol, the deterioration in performance during the exposure was greater, and performance improved after the exposure ended. This effect was not due to preexposure differences; 10 of the 12 subjects showed greater performance decrements during methanol than during sham exposure. The effects of exposure did not differ as a function of single versus dual task performance.

Response time in the Sternberg memory task is a function of three cognitive processes: (1) stimulus perception and encoding, (2) memory scanning, and (3) response selection and execution. The times required to perform the first and third processes are indexed by the y-intercept measure. Changes in the y-intercept indicate changes in the perceptual or the response aspects of task performance. The slope measure is considered to be a direct index of the time required to search the memory store. Multivariate analysis of the slope and intercept revealed a trend for an exposure-by-testing-period interaction (Wilk's lambda = 0.624, F = 2.52, df 4,38, p = 0.057); neither of the variables had a univariate exposure-by-period interaction, indicating that exposure affected the relationship between the slope and the intercept. As shown in Figure 12, the intercept increased as the slope decreased on sham exposure days. When subjects were exposed to methanol, there was no orderly relationship between the slope and the intercept. Reaction time, slope, and intercept measures, therefore, all suggest that methanol exposure might have some deleterious effects on the ability to search the memory store quickly.

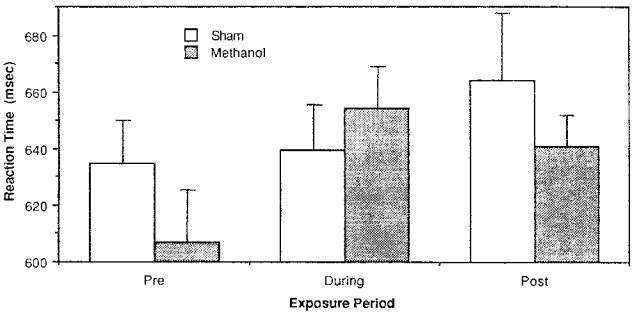


Figure 11. Performance on the Sternberg memory task. Greater deterioration in reaction time is shown when subjects were exposed to methanol at 250 mg/m^3. Recovery occurred after the exposure ended. Error bars for the preexposure testing period are the standard error of the mean; error bars for the exposure and postexposure testing periods are the standard error of the change from the preexposure testing period.

The interval production task was performed alone and together with the speeded addition task; the interval production task was designated the primary task. As expected, subjects had higher interval production task scores (poorer performance) under dual task conditions (F = 24.30, df 1,10, MSE = 6.96, p = 0.001). There was also a trend toward higher interval production task scores when subjects were exposed to methanol than when exposed to sham vapors (F = 3.64, df 1,10, MSE = 7.22, p = 0.09), and this effect interacted with the order of exposure (F = 4.34, df 1,10, MSE = 0.40, p = 0.064). However, no exposure-by-testing-period interaction was observed, suggesting that the differences were not due to exposure to methanol vapor.

Performance on the secondary speeded addition task, as expected, was poorer under dual task conditions (F = 8.52, df 1,10, MSE = 2.93, p = 0.02). A complex interaction be-

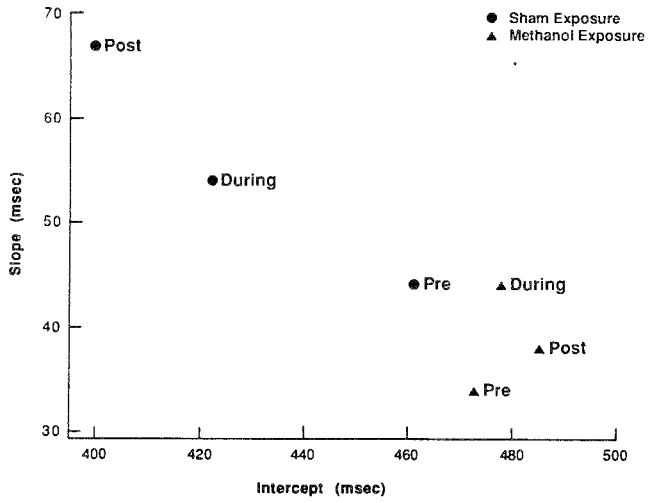


Figure 12. Scatterplot of the relationship between the slope and intercept of the Sternberg memory task under methanol and sham exposure conditions. The strong negative correlation seen under sham conditions is not seen when subjects are exposed to methanol at 250 mg/m^3.

tween exposure, testing period, single versus dual tasks, and order of exposure to methanol and sham vapors was found (F = 3.97, df 2,20, MSE = 1.52, p = 0.05). When the two order groups were analyzed separately, no exposure effect was found for either group.

PHYSIOLOGICAL MEASURES

No significant effects of methanol exposure were found for cardiac interbeat interval, respiration, the spectral analysis of the EEG or the contingent negative variation. However, these variables did show the expected changes as a function of time of day, as well as the expected relationships among the variables. Because no change in cardiac interbeat interval was observed, the components of the ECG were not submitted to statistical analysis.

Auditory-Event-Related Potential

Presentation of a stimulus will evoke transient electrophysiological responses in the human brain. Computer averaging of such responses recorded at the scalp results in a complex waveform with distinct electrically positive and negative components. Components are usually identified sequentially by their typical time of occurrence after the stimulus, and by whether or not they are electrically positive or negative. The four most commonly studied cognitive components are N100, P200, N200, and P300 (a negative wave occurring at about 100 msec after the stimulus, a positive wave occurring about 200 msec after the stimulus, and so forth).

A large body of research has attempted to relate changes in the amplitude and latency of these components to perceptual and cognitive processes. The relationships found are not always perfect, and disagreement exists about the functional significance of different components; nevertheless, there is general consensus that specific components provide indices of particular aspects of human information processing activities. For example, N100 is involved in the initial process of stimulus registration. N200 is similar to a "mismatch" detector and indexes stimulus classification activities. The P200 component is not as well understood as the others, but may reflect processes involved in comparing incoming stimuli with neuronal models. The P300 component of the event-related potential is the most widely studied. Numerous studies indicate that this component is intimately related to higher-order stimulus evaluation and decision-making activities.

The amplitude and latency of the components of the event-related potential can be analyzed in two ways. The absolute amplitude, as compared to a sample of EEG activity recorded before stimulus presentation, can be calculated, as well as the absolute latency between the peak of the component and the point at which the stimulus was presented. Peak-to-trough amplitude and latency also can be calculated. These measures are usually referred to as N1P2, N2P3, and so forth.

It was not possible to include all event-related potential measures in one multivariate analysis. Consequently, separate analyses were performed for the auditory- and visual-event-related potentials, and within these categories, absolute amplitude, absolute latency, peak-to-trough amplitude, and peak-to-trough latency were analyzed separately.

No significant multivariate effects involving exposure to methanol were found for the absolute amplitude or the peak-to-trough amplitude of the auditory-event-related potential, although the expected changes as a function of target and nontarget stimuli, and of time of day, were observed.

The latency of the auditory-event-related potential yielded significant multivariate exposure effects for absolute latency that arose from the P200 component (Wilk's lambda = 0.369, F = 2.75, df 8,34, p = 0.02), and differed as a function of order of exposure (Wilk's lambda = 0.404, F = 2.44, df 8,34, p = 0.034). The univariate analyses, therefore, were examined. The latency of the P200 component decreased across the experimental session when subjects were exposed to sham vapors; when they were exposed to methanol, latency was longer during the actual exposure (interaction F = 7.65, df 2,20, MSE = 105.28, p = 0.01). The interaction is shown in Figure 13. The magnitude of the effect was greater for those subjects who were exposed in the

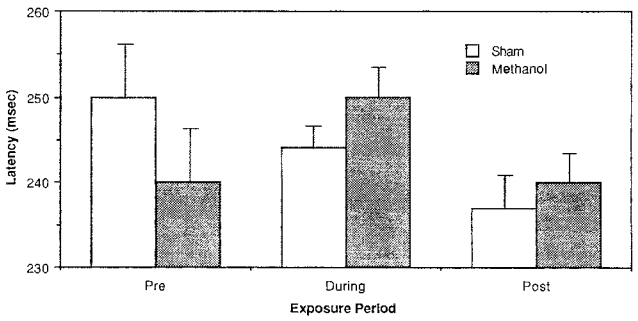


Figure 13. Latency of the P200 component of the auditory-event-related potential. Latency decreased across the experimental session when subjects were exposed to sham vapors. When they were exposed to methanol at 250 mg/m^3, latency was longer during the actual exposure. Error bars for the preexposure testing period are the standard error of the mean; error bars for the exposure and postexposure testing periods are the standard error of the change from the preexposure testing period.

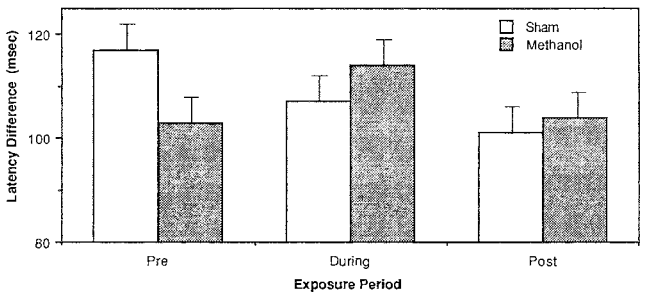


Figure 14. The time difference between the N100 and P200 components of the auditory-event-related potential. The increased elapsed time between the two components is shown as a function of exposure to methanol at 250 mg/m^3. Error bars for the preexposure testing period are the standard error of the mean; error bars for the exposure and postexposure testing periods are the standard error of the change from the preexposure testing period.

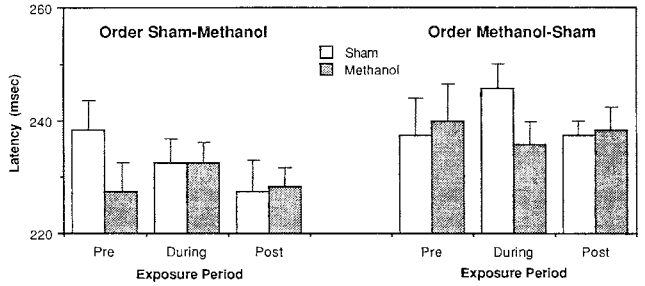


Figure 15. The latency of the P200 component of the visual-event-related potential. For subjects exposed in the order sham-methanol, the pattern is similar to that shown in Figure 14 for the auditory task; for those exposed in the order methanol-sham, it is not. Error bars for the preexposure testing period are the standard error of the mean; error bars for the exposure and postexposure testing periods are the standard error of the change from the preexposure testing period.

order methanol-sham than for those exposed in the order sham-methanol (interaction F = 8.24, df 2,20, MSE = 105.28, $p < 0.01$). These effects cannot be attributed entirely to preexposure differences over experimental sessions. Of the 12 subjects, 8 had greater increases in P200 latency between preexposure and exposure testing periods in methanol exposures than in sham exposures.

Similar effects were found for peak-to-trough latency, and are shown in Figure 14. The time between N1 and P2 decreased across the experimental session when subjects were exposed to sham vapors, but increased during exposure to methanol vapors (interaction F = 5.61, df 2,20, MSE = 256.39, p = 0.01), and again, the magnitude of the effect was greater for subjects exposed in the order methanol-sham (interaction F = 4.05, df 2,20, MSE = 256.39, p = 0.04).

Visual-Event-Related Potentials

Data for visual-event-related potential amplitudes were similar to those for the auditory task; no multivariate effects of exposure were found for either absolute or peak-to-trough measures of amplitude. However, significant multivariate interactions between exposure, testing period, and order of exposure were found for absolute latency (Wilk's lambda = 0.374, F = 2.70, df 8,34, p = 0.02) and peak-to-trough latency (Wilk's lambda = 0.394, F = 3.56, df 6,36, $p < 0.01$), and arose from P200 and N1P2. Univariate analyses for these variates were therefore interpreted. Significant exposure-by-testing-period-by-order-of-exposure interactions were found for P200 (F = 4.73, df 2,20, MSE = 86.94, p = 0.02) and for N1P2 (F = 9.10, df 2,20, MSE = 105.97, p = 0.003). This effect is illustrated for P200 in Figure 15. The patterns for N1P2 were the same.

For the group exposed in the order sham-methanol, the patterns were quite similar to those seen in the auditory-event-related potential: latency decreased during the sham exposure, and increased during the methanol exposure. The patterns for the methanol-sham exposure group, however, were quite unlike those for the auditory-event-related potential. The patterns observed suggest that P200 latency in the visual task was different on the first and second experimental days, regardless of whether or not the subject was exposed to methanol during the experimental session.

REGRESSION ANALYSIS TO PREDICT CHANGES IN DEPENDENT VARIABLES

If methanol exposure actually alters performance, physiological, or subjective variables, one would expect that larger changes in blood levels of methanol would be associated with larger changes in the dependent variables, and that this might be influenced by such factors as the individual's body weight, the concentration of methanol in the test room, and the subject's ability to detect the presence of methanol. The final set of analyses was performed to ex-

plore these relationships. In the present study, the range of room methanol concentrations and the range of changes in blood levels of methanol were quite small. Under such circumstances, only very robust relationships would be expected to be revealed by the analyses.

Regressions were calculated for six variables: fatigue, concentration, and vigor scales from the Mood Adjective Check List; latency of P200 to target and nontarget stimuli from the auditory-event-related potential; and reaction time from the Sternberg memory task. For each variable, two regressions were calculated. One, based on the changes from the preexposure testing period to the exposure testing period, is labeled the exposure effect. The other, based on changes from the exposure testing period to the postexposure testing period, is labeled the recovery effect.

The predictor variables for the regressions were the concentration of methanol in the room, change in blood methanol concentration, postexposure blood methanol concentration, body weight, and the number of times the subject was correct in his double-blind judgments on methanol exposure days.

The results are summarized in Table 11. For the fatigue scale score, there were no predictors for the exposure effect with probability of 0.10 or less; the recovery effect was best predicted by the number of correct double-blind judgments. For the concentration score, the exposure effect was best predicted by postexposure blood methanol levels. Sixty percent of the variance in the recovery effect was predicted by three variables: the number of correct double-blind judgments, the change in blood methanol, and the concentration of methanol in the exposure room. None of the predictor variables significantly predicted the exposure effect for the vigor scale, but two variables predicted 40 percent of the variance in the recovery effect: change in blood methanol concentrations, and the number of correct double-blind judgments.

Latency of P200 to auditory target stimuli was best predicted by room methanol concentration and by postexposure blood methanol, and this was true for both the exposure effect and the recovery effect. Latency to nontarget stimuli was best predicted by room methanol concentration for the exposure effect; none of the variables predicted the recovery effect. Finally, performance on the Sternberg memory task was not significantly predicted by any of the variables.

The number of correct double-blind judgments was often a significant predictor of subjective mood scale scores. Further research should include measures that would clarify the extent to which changes in mood, which are typically easily identified by subjects, are used by individual subjects to guide their double-blind judgments.

The results of these regression analyses suggest that if more than one level of methanol concentration were used, meaningful dose-response relationships might be found even at levels of methanol exposure expected as a result of its use as a motor fuel.

Table 11. Summary of Regression Analyses to Predict Changes in Dependent Variables

		Exposure Effect[a]					Recovery Effect[a]			
Dependent Variable	Predictor Variable	R^2	p	ΔR^2	p	Predictor Variable	R^2	p	ΔR^2	p
Concentration	Blood methanol postexposure	0.26	0.09			Double-blind no. correct	0.30	0.07		
						Blood methanol change	0.45	0.07	0.15	0.16
						Room methanol	0.60	0.05	0.16	0.12
Vigor		No predictors at $p < 0.10$				Blood methanol change		0.27		0.08
						Double-blind no. correct	0.40	0.10	0.13	0.19
Sternberg memory task slope	Weight	0.53	0.01				No predictors at $p < 0.10$			
	Double-blind no. correct	0.59	0.02	0.06	0.26					
	Room methanol	0.61	0.05	0.02	0.53					
	Blood methanol change	0.63	0.10	0.01	0.64					
Fatigue		No predictors at $p < 0.10$				Double-blind no. correct	0.26	0.09		
P200 latent targets	Room methanol	0.22	0.12			Room methanol	0.19	0.15		
	Blood methanol postexposure	0.42	0.08	0.20	0.11	Blood methanol postexposure	0.58	0.02	0.39	0.02
P200 latent nontargets	Room methanol	0.31	0.06				No predictors at $p < 0.10$			
Sternberg memory task reaction time		No predictors at $p < 0.10$					No predictors at $p < 0.10$			

[a] R^2 is the squared multiple correlation coefficient.

DISCUSSION

The major objective of the work reported here was to perform a controlled pilot study of the effects of exposure to low levels of methanol vapor on many measures of human function important in daily living. In this section of the report, we discuss the results of the study, relate them to other work, point out the limitations of the study, and make recommendations for future research.

METHANOL AND FORMATE LEVELS IN BODY FLUIDS

Methanol is rapidly absorbed by inhalation, ingestion, and dermal exposure and distributes uniformly in aqueous body fluids. It is eliminated from the body primarily by metabolism, but is also excreted via kidneys and lungs. Elimination of methanol in humans exposed to 100 or 300 ppm in air appears to follow zero-order kinetics with a half-life of one to three hours (Sedivec et al. 1981; National Research Council 1985). Dutkiewicz and coworkers (1980) reported that humans experimentally exposed to methanol dermally and by inhalation excreted maximal amounts of methanol in urine within four hours after exposure.

As expected in this study, exposure to methanol at 250 mg/m^3 for 75 minutes markedly increased the concentration of methanol in blood and urine. (The pattern of change observed, together with the available literature [for example, Benoit et al. 1985], supports the conclusion that subjects in this study had elevated methanol levels that were probably relatively constant throughout the entire exposure testing period.) Although methanol concentrations before exposure were higher in urine than in blood (47 of 48 comparisons), the changes as a function of exposure were greater in blood (20 of 24 comparisons). This experiment was not designed to examine the half-life of methanol, although it did provide some interesting data. Urinary methanol concentration was measured immediately after exposure, and again at the end of the experimental session (about one hour after exposure ended). Within this postexposure testing period, methanol concentration decreased 11 times, and increased 13 times.

There was no systematic relationship between the concentration of methanol in the test room and blood or urine methanol levels (either absolute values at the end of the exposure or percent change from preexposure to postexposure), probably because of the restricted range of methanol concentration in the test room. The data on methanol in body fluids obtained in this study are in good agreement with previous research (for example, Sedivec et al. 1981).

The major metabolic route for methanol in primates is oxidation, via an alcohol dehydrogenase enzyme system, to formaldehyde, which is eliminated rapidly and completely by the body, primarily by metabolism to formic acid. In primates, formic acid is oxidized slowly to carbon dioxide via the folate biochemical pathway. Accumulation of formic acid in blood causes metabolic acidosis, and plasma concentrations of formate have been correlated with the ocular toxicity characteristic of methanol poisoning in primates (Tephly and McMartin 1984).

Issues related to urinary concentration of formate have been reviewed by Boeniger (1987). Formate concentration would be expected to be higher than the concentration of methanol after exposure, but this should not occur until significant metabolism has taken place. In fact, Liesivuori and Savolainen (1987) found no correlation between urinary formate and exposures of workers to 40 to 160 ppm methanol until 16 hours after the end of the work day. At that time, formate levels were two to seven times higher than the urine concentrations of unexposed individuals.

Plasma formate levels were not increased by exposure in the present study, and this finding is consistent with the fact that urinary levels of methanol were still elevated at the end of the experimental session. Consistent with the lack of increase in formate levels, no changes in visual function were observed. Vital signs and subjective health symptoms were also unaffected by exposure. These findings support the hypothesis that methanol accumulation from brief exposures to the ambient levels of methanol predicted by traffic scenarios should not exceed the capability of healthy humans to oxidize the incremental formate resulting from methanol metabolism (Health Effects Institute Special Report 1987).

NEUROBEHAVIORAL EFFECTS

In previous neurobehavioral research, the lowest effective levels of inhaled methanol in humans were reported by Chao (1959) and Ubaydullayev (1968), who found changes in light sensitivity and conditioned brain responses. These reports are difficult to interpret; the number of subjects used was small, and the effects were not consistent between the two reports. To our knowledge, all other methanol studies in humans were occupational studies. Headache and changes in information processing rate, muscular work capacity, and mood have been reported (see reviews by Carson et al. 1981, 1987). However, these studies are not particularly helpful because of lack of controls, poor definition of endpoints, and frequent confounding of exposure by the presence of other potential toxicants.

Few of the neurobehavioral test battery items were affected by exposure to methanol in this study. Even for those tests in which deleterious effects were observed, the data

were within the normal range. Thus, as expected, effects of exposure to methanol at 250 mg/m^3 were few, small, and subtle. However, the apparent effects observed form a consistent and interesting pattern.

When they were exposed to methanol, subjects reported higher levels of fatigue and there was a trend toward poorer concentration and less vigor. The implications of such changes for driving are obvious; however, the absolute magnitude of the changes was quite small, and did not appear to affect the subjects' ability to maintain vigilance or to respond quickly to stimuli. It is possible that, had a more sensitive measure of workload been used, subjects would have reported that they worked harder in order to maintain simple task performance when they were exposed to methanol. Consistent with increases in fatigue and decreases in vigor and concentration, subjects showed changes in their ability to perform the Sternberg memory task.

The latency of the P200 component of the event-related potential was also increased during methanol exposures. Most studies of the event-related potential have focused on the very early exogenous components (occurring in the first 50 msec) that reflect sensory processes, or on the later endogenous components (occurring after 200 msec) that reflect cognitive processes. Most of the work has attempted to define the circumstances under which changes in the amplitude and latency of N200 and P300 occur. Mismatch negativity, defined as a marked increase in the amplitude of N200, is obtained when an infrequent stimulus is presented in a chain of frequent stimuli. Mismatch negativity occurs regardless of whether or not the subject is attending to the stimuli, and it is specific to modality in its scalp distribution (Näätänen 1982). The P300 component occurs when a task-relevant, infrequent stimulus occurs, and its scalp distribution is not dependent on modality (Donchin 1981, 1984). Both the Sternberg memory task (for example, Pratt et al. 1989) and the Stroop color-word test (for example, Duncan-Johnson 1981) have been used as experimental paradigms for studying N200 and P300.

The P200 component has been largely unstudied because it falls between the sensory and cognitive categories. Picton (1980) has suggested that the "mesogenous" components of the event-related potential—those after the clear sensory components, but before those that are associated almost exclusively with cognitive processes—may reflect a general comparative process in which sensory information is related to endogenous memories before being interpreted by the subject. If Picton's hypothesis is correct, the changes observed in P200 latency in the present study are consistent with the performance changes observed in the Sternberg memory task. This interpretation is supported by the observation that P200 latency and reaction time on the Sternberg memory task were both best predicted by the concentration of methanol in the test chamber. On the other hand, P200 appears remarkably resistant to either sensory or cognitive manipulation, and the results reported here, if replicated, might imply specific neurophysiological effects of exposure to low levels of methanol.

LIMITATIONS OF THE PRESENT STUDY

The study had three major limitations. First, no data on which to base sample size were available, and sample size was determined by the investigators' previous experience with the tasks in the battery. Some of the items in the battery yielded results that approached statistical significance. In future work, sample size should be increased. For example, the probability of the exposure effect on the Sternberg memory task was 0.055, with adjusted degrees of freedom of 1.76 and 17.55. This suggests that, if the same design were to be used in a verification study, sample size should be increased to at least 18.

A second limitation was that the attempt to perform the study under double-blind control conditions was not entirely successful. The experimenter's judgment of the presence of methanol did not appear to affect the subjects' judgments. However, some subjects judged the presence of methanol at better-than-chance levels. Given the small sample size, it is impossible to determine with any confidence if the subjects' opinions about whether or not they were being exposed to methanol actually affected the dependent measures. As discussed below, the group most accurate in judging the presence of methanol did not show greater changes in neurobehavioral test battery items. This suggests that subjects' performance was not influenced by subjective opinion regarding the presence or absence of methanol.

A third limitation of the study was that only one level of methanol exposure and only one exposure duration were tested, making it impossible to determine whether or not there was a dose-response effect for the variables that appeared to be affected by exposure to methanol.

In studies in which subjects serve as their own controls, conditions are usually counterbalanced so that significant results cannot be attributed to the order in which the subjects were exposed to the experimental treatment. Often it is assumed that counterbalancing is successful, and no specific tests for order effects are included in data analysis. In the present study, order effects were specifically tested, and differences between subjects exposed in the order sham-methanol and those exposed in the order methanol-sham were found for many of the dependent variables examined. These differences do not appear to arise from the difference between the two groups in judging the presence of metha-

nol. Subjects exposed first to methanol were more accurate in their judgments than those exposed first to sham vapor. Exposure effects, however, tended to occur more frequently for subjects exposed first to sham vapor. The order effects observed are not uncommon in designs of this type, and they appear to be due to chance individual differences between the two groups. Using a larger sample size would have reduced the likelihood of finding such chance group differences.

RECOMMENDATIONS

Most of the variables tested in the present study did not appear to be affected by exposure to methanol at 250 mg/m^3 for 75 minutes; however, some significant differences and trends were found. Although the changes were subtle and within the normal range for healthy young men, they suggest that further research should be carried out. First, the number of changes observed was relatively small, and may have been due to chance. Only a study designed to verify and extend the findings can address this question. For such a study, we would make the following recommendations.

- Employ a larger sample size of at least 18 subjects.
- Include all variables for which exposure effects with probability less than 0.20 were found in the present experiment (visual- and auditory-event-related potentials, systolic blood pressure, cardiac interbeat interval, Sternberg memory task, Stroop color-word test, Symbol Digit substitution task, critical flicker fusion, near acuity and near lateral phoria, and fatigue and mood scales).
- Use odor-masking techniques to reduce the probability that either subjects or experimenters will be able to detect the presence of methanol.
- Include some more difficult tasks to determine whether or not effects are, as suggested by present results, in part a function of the difficulty or complexity of the task.
- Because there is no suggestion that exposure to methanol improves performance or physiological function, increase the training to more stringent criteria to reduce variability across subjects.
- Specifically test blood alcohol levels before exposure.
- Quantify urinary levels of methanol and formate several hours after exposure, and perform tests of visual function at the same point in time.
- Make minor alterations in the facility to provide better control over environmental conditions.

In addition to a verification study, other research is needed in order to provide adequate data for risk assessment. The following recommendations are made for such work.

- Perform a human study that systematically varies both the concentration of methanol and the duration of exposure and compares effects of exposure to methanol with effects of exposure to gasoline vapors.
- Systematically alter ventilation to see if exercising subjects (for example, those jogging or bicycling near major traffic areas) would be at greater risk of exposure effects.
- Obtain better human pharmacokinetic data, including the time course of changes in the body burden of formaldehyde, methanol, and formate.
- Perform nonhuman primate neurobehavioral studies to examine the effect of chronic exposure on variables shown to be acutely affected in humans.
- It is possible that methanol may interact with other toxicants (for example, Aarstad et al. 1984; Kushneva et al. 1986); consequently, there is a need to explore the effects of mixtures (for example, methanol plus aromatics, olefins, odorants, or alcohol) on human neurobehavioral function.

The study reported here has provided important preliminary data about the effects of acute exposure to a level of methanol that might be encountered if methanol were used as a fuel for motor vehicles. Exposure did not result in adverse affects on health; however, some physiological, performance, and subjective variables did appear to be affected in this sample of healthy young men exposed for only 75 minutes. It is possible that larger or more serious neurobehavioral effects might be observed after longer exposure to methanol, or in special populations, such as the elderly, pregnant women, or individuals with health problems. Further research is needed to provide the data required for risk assessment, and to compare the neurobehavioral effects of methanol with those of other motor fuels. There is a need for further research to verify and extend the results reported here, and to examine further their implications for adequate risk assessment.

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ing the methanol analyses; Debbie Alexander, for collecting blood samples; and James W. Phelps and Joe Fessler, for computer programming. The authors wish to thank an anonymous reviewer for suggesting the recovery analysis.

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APPENDIX A. Study of Perception of Methanol Vapor at 250 mg/m^3

Little reliable information is available about the ability of humans to detect the presence of methanol vapor, and the threshold values in the literature vary widely, from 3.4 ppm (Ubaydullayev 1968) to 7,800 ppm (May 1966). It is difficult to reconcile such wide differences, even allowing for different experimental techniques. Project staff were, therefore, concerned that both subjects and experimenters might be able to detect the odor of methanol. When test room construction was complete and the system for introducing methanol vapor into the test room had been verified, the Principal Investigator and two other senior members of the project team attempted to identify cues to the presence of methanol vapor at the concentration to be used in the planned study. Despite the fact that they were aware of the actual conditions, they were unable to do so. Subsequently, two preliminary studies were conducted to provide more complete data with regard to cues that might compromise the double-blind design of the study. The study protocols for these preliminary studies were reviewed by the MRI Human Subjects Committee, and all test volunteers provided written informed consent prior to participation.

Five MRI staff members participated in the first preliminary study. Each subject entered the test room twice and on each occasion remained there for 10 minutes. On one occasion methanol vapor (250 mg/m^3) was present, and on the other sham vapor was present. The subjects knew that, at some time during their participation, methanol would be present in the test room. Their task was to judge, using all sensory information available to them, whether or not methanol was present when they first entered the test room or when they left the test room. For each judgment, they were required to give a confidence rating and to explain why they reached their decision.

Under sham conditions, all five subjects judged that methanol vapor was not present, either when they entered the test room or when they left it. When methanol vapor was present in the test room, two subjects made correct judgments about the conditions when they entered the test room; however, both said that methanol was not present when they left the test room, and both reported very low confidence in the judgment. The level of methanol in the room was not changed during these exposures.

Six MRI employees with backgrounds in chemistry or environmental science participated in the second preliminary study. All met criteria for entry into the planned experiment (male nonsmokers, 21 to 35 years of age), and all reported that they were familiar with the odor of methanol. These subjects were paid $10 each for their participation. The design was similar to that used for the first preliminary study, except that subjects entered the test room four times and remained there for only five minutes each time. Each subject was exposed to methanol vapor twice and to sham vapor twice.

Table A.1 summarizes the results of the second preliminary study. Judgments did not differ significantly from those expected due to chance. When methanol vapors were present, 38 percent of the judgments were correct; when methanol was not present, 62 percent of the judgments were correct. The 18 judgments in which the subjects had high confidence are particularly interesting: 16 judgments were that methanol was not present; 6 of these were correct. Subjects were highly confident that methanol was present on only two occasions; both judgments were incorrect. Thus, when subjects familiar with the odor of methanol were highly confident of their judgments, they were right only 6 of 18 times. Experience in the exposure room did not improve perception. Subjects were no more accurate in their last two exposure sessions than they were in the initial two exposure sessions. These data (Table A.1) supported the conclusion that the experiment could be conducted under double-blind conditions without masking the odor of methanol and without further changes in the equipment to mask sounds or other cues that might give the subjects or the experimenters information about the presence or absence of methanol.

Table A.1. Results of Subjects' Attempts to Judge Whether Methanol Vapors Were Present or Absent

	Methanol Was	
Judged	Present	Absent
Day 1		
Present	4	3
Absent	8	9
Day 2		
Present	5	6
Absent	7	6

APPENDIX B. Concentrations of Methanol in Blood and Urine, and of Formate in Plasma

Table B.1. Concentrations of Methanol in Blood and Urine[a] for Each Subject During Sham and Methanol Exposures

Subject	Body Fluid	Exposure	Data Collection Period			Percent Change	
			Preexposure	Postexposure	End of Experimental Session	Post – Pre	End – Post
01	Blood	Sham	0.51	0.46		– 9.8	
		Sham	0.39	0.44		+ 12.8	
		Methanol	0.26	1.66		+ 538.5	
		Methanol	0.27	1.28		+ 374.1	
	Urine	Sham	0.63	0.59	0.53	– 6.3	– 10.2
		Sham	0.78	0.82	0.66	+ 5.1	– 19.5
		Methanol	0.65	1.68	1.56	+ 158.5	– 7.1
		Methanol	1.03	2.49	1.83	+ 141.7	– 26.5
02	Blood	Sham	0.33	0.37		+ 12.1	
		Sham	0.28	0.23		– 17.9	
		Methanol	0.58	1.62		+ 179.3	
		Methanol	0.51	1.66		+ 225.5	
	Urine	Sham	0.87	0.96	0.78	+ 10.3	– 18.8
		Sham	0.75	0.91	0.54	+ 21.3	– 40.7
		Methanol[b]	–	–	–	–	–
		Methanol	0.86	2.26	1.96	+ 162.8	– 13.3
03	Blood	Sham	0.56	0.49		– 12.5	
		Sham	0.29	0.20		– 31.0	
		Methanol	0.45	1.69		+ 275.6	
		Methanol	0.37	2.10		+ 467.6	
	Urine	Sham	1.08	0.90	0.74	– 16.7	– 17.8
		Sham	0.91	0.85	1.08	– 6.6	+ 27.1
		Methanol	0.88	2.27	2.16	+ 158.0	– 4.8
		Methanol	0.76	2.38	1.89	+ 213.2	– 20.6
04	Blood	Sham	0.85	0.63		– 25.9	
		Sham	0.94	0.75		– 20.2	
		Methanol	1.14	2.50		+ 119.3	
		Methanol	0.56	1.76		+ 214.3	
	Urine	Sham	1.44	1.28	1.13	– 11.1	– 11.7
		Sham	1.38	1.52	1.48	+ 10.1	– 2.6
		Methanol	1.48	2.92	3.02	+ 97.3	+ 3.4
		Methanol	1.19	2.99	3.52	+ 151.3	+ 17.7
05	Blood	Sham	0.66	0.78		+ 18.2	
		Sham	0.58	0.40		– 31.0	
		Methanol	0.76	1.57		+ 106.6	
		Methanol	0.25	1.27		+ 408.0	
	Urine	Sham	1.00	0.88	0.85	– 12.0	– 3.4
		Sham	1.63	1.28	1.14	– 21.5	– 10.9
		Methanol	1.32	2.12	2.59	+ 60.6	+ 22.2
		Methanol	0.81	1.47	1.76	+ 81.5	+ 19.7
06	Blood	Sham	0.45	0.39		– 13.3	
		Sham	0.90	0.62		– 31.1	
		Methanol	0.48	1.89		+ 293.8	
		Methanol	0.34	1.46		+ 329.4	
	Urine	Sham	1.09	1.18	0.97	+ 8.3	– 17.8
		Sham	1.26	1.52	1.12	+ 20.6	– 26.3
		Methanol	0.84	2.23	2.08	+ 165.5	– 6.7
		Methanol	0.81	2.03	1.94	+ 150.6	– 4.4

(*Table continues next page.*)

Table B.1. (*continued*)

Subject	Body Fluid	Exposure	Data Collection Period: Preexposure	Data Collection Period: Postexposure	Data Collection Period: End of Experimental Session	Percent Change: Post – Pre	Percent Change: End – Post
07	Blood	Sham	0.40	0.27		– 32.5	
		Sham	0.35	0.37		+ 5.7	
		Methanol	0.36	1.40		+ 288.9	
		Methanol	0.30	2.14		+ 613.3	
	Urine	Sham	0.67	0.61	0.54	– 9.0	– 11.5
		Sham	0.73	0.63	0.63	– 13.7	0
		Methanol	0.49	1.43	1.62	+ 191.8	+ 13.3
		Methanol	0.47	1.63	1.80	+ 246.8	+ 10.4
08	Blood	Sham	0.29	0.25		– 13.8	
		Sham	0.54	0.50		– 7.4	
		Methanol	0.39	1.89		+ 384.6	
		Methanol	0.59	2.22		+ 276.3	
	Urine	Sham	0.81	0.86	0.71	+ 6.2	– 17.4
		Sham	0.59	0.58	0.51	– 1.7	– 12.1
		Methanol	0.57	1.88	2.23	+ 229.8	+ 18.6
		Methanol	0.90	2.12	2.43	+ 135.6	+ 14.6
09	Blood	Sham	0.36	0.35		– 2.8	
		Sham	0.49	0.46		– 6.1	
		Methanol	0.49	– [c]	– [c]		
		Methanol	0.37	1.71		+ 362.2	
	Urine	Sham	0.63	0.60	0.61	– 4.8	+ 1.7
		Sham	0.91	0.80	0.73	– 12.1	– 8.8
		Methanol	0.60	1.42	1.65	+ 136.7	+ 16.2
		Methanol	0.59	1.42	1.85	+ 140.7	+ 30.3
10	Blood	Sham	0.51	0.37		– 27.5	
		Sham	1.51	1.26		– 16.6	
		Methanol	0.47	1.44		+ 206.4	
		Methanol	0.54	1.88		+ 248.1	
	Urine	Sham	0.68	0.55	0.52	– 19.1	– 5.5
		Sham	1.99	1.92	1.44	– 3.5	– 25.0
		Methanol	0.54	1.94	1.78	+ 259.3	– 8.2
		Methanol	0.47	2.03	1.87	+ 331.9	– 7.9
11	Blood	Sham	0.94	0.96		+ 2.1	
		Sham	1.17	1.47		+ 25.6	
		Methanol	1.40	2.50		+ 78.6	
		Methanol	1.17	3.20		+ 173.5	
	Urine	Sham	1.20	1.15	1.10	– 4.2	– 4.3
		Sham	1.43	1.75	1.56	+ 22.4	– 10.9
		Methanol	1.89	3.81	4.14	+ 101.6	+ 8.7
		Methanol	1.34	2.63	4.02	+ 96.3	+ 52.9
12	Blood	Sham	0.46	0.48		+ 4.3	
		Sham	0.74	0.72		– 2.7	
		Methanol	0.80	2.65		+ 231.2	
		Methanol	0.82	1.95		+ 137.8	
	Urine	Sham	1.03	0.97	1.04	– 5.8	+ 7.2
		Sham	1.03	1.19	0.93	+ 15.5	– 21.8
		Methanol	0.93	2.66	2.39	+ 186.0	– 10.2
		Methanol	1.04	2.63	2.69	+ 152.9	+ 2.3

[a] Methanol concentrations are given in milligrams per liter.
[b] Missing data; suspected analysis error.
[c] Missing data.

Table B.2. Concentrations of Plasma Formate[a] for Each Subject Before and After Exposure to Methanol

Subject	Exposure	Pre	Post	Percent Change
01	Sham	0.074	0.071	- 4.0
	Sham	0.080	0.075	- 6.2
	Methanol	0.072	0.075	+ 4.2
	Methanol	0.047	0.050	+ 6.4
02	Sham	0.047	0.109	+ 131.9
	Sham	0.073	0.071	- 2.7
	Methanol	0.124	0.098	- 21.0
	Methanol	0.069	0.079	+ 14.5
03	Sham	0.060	0.040	- 33.3
	Sham	0.058	0.102	+ 75.9
	Methanol	0.117	0.152	+ 29.9
	Methanol	0.078	0.105	+ 34.6
04	Sham	0.098	0.099	+ 1.0
	Sham	0.059	0.044	- 25.4
	Methanol	ND[b]	0.086	ND
	Methanol	0.049	0.060	+ 22.4
05	Sham	0.055	0.069	+ 25.4
	Sham	0.110	0.095	- 13.6
	Methanol	0.104	0.110	+ 5.8
	Methanol	0.056	0.050	- 10.7
06	Sham	0.062	0.051	- 17.7
	Sham	0.077	0.105	+ 36.4
	Methanol	0.096	0.073	- 24.0
	Methanol	0.115	0.096	- 16.5
07	Sham	0.090	0.087	- 3.3
	Sham	0.117	0.093	- 20.5
	Methanol	ND	0.071	ND
	Methanol	0.109	0.086	- 21.1
08	Sham	0.071	0.053	- 25.4
	Sham	0.086	0.083	- 3.5
	Methanol	0.100	0.057	- 43.0
	Methanol	0.067	0.060	- 10.4
09	Sham	0.101	0.093	- 7.9
	Sham	ND	0.063	ND
	Methanol	0.073	0.071	- 2.7
	Methanol	0.049	0.078	+ 59.2
10	Sham	0.070	0.062	- 11.4
	Sham	0.049	0.055	+ 12.2
	Methanol	0.067	0.059	- 11.9
	Methanol	0.104	0.081	- 22.1
11	Sham	0.033	0.033	0.0
	Sham	0.143	0.125	- 12.6
	Methanol	0.053	0.064	+ 20.8
	Methanol	0.068	0.044	- 35.3
12	Sham	0.080	0.044	- 45.0
	Sham	0.117	0.114	- 2.6
	Methanol	0.102	0.086	- 15.7
	Methanol	0.074	0.070	- 5.4

[a] Formate concentrations are given in millimoles per liter.
[b] ND = not determined.

APPENDIX C. Statistics on the Exposure-by-Testing-Period Interaction for Each Dependent Variable

For each of the dependent measures obtained in the study, the following tables present the mean values for methanol and sham exposures, the *F* values, the degrees of freedom, the mean-squared errors, and the probability values corrected for sphericity. Table C.1 is devoted to measures obtained in all experimental sessions. Table C.2 presents data from battery A, and Table C.3 contains data from battery B. The information contained in this appendix should be helpful to investigators planning future research on the effects of methanol on human function.

Table C.1. Statistics for Dependent Variables Obtained in All Experimental Sessions

Variable	Units	Exposure	Means			F	df	MSE	Corrected p
			Pre	During	Post				
Blood methanol	mg/L	Sham	0.604	—	0.551				
		Methanol	0.570	—	1.881	583.33	1,10	0.019	< 0.001
Urinary methanol	mg/L	Sham	1.022	1.012	0.889				
		Methanol	0.888	2.196	2.281	124.43	2,20	0.066	< 0.001
Plasma formate	mmol/L	Sham	0.0785	—	0.0765				
		Methanol	0.0820	—	0.0775	0.16	1,10	0.0002	0.70
Oral temperature	°F	Sham	97.7	97.6	97.4				
		Methanol	97.5	97.6	97.3	0.17	2,20	0.20	0.77
Systolic blood pressure	mm Hg	Sham	119.2	114.9	112.7				
		Methanol	114.9	114.2	111.3	2.52	2,20	81.18	0.12
Diastolic blood pressure	mm Hg	Sham	76.0	74.4	75.7				
		Methanol	74.7	74.8	74.2	0.58	2,20	19.56	0.55
Pulse rate	Beats per minute	Sham	65.5	60.8	57.9				
		Methanol	66.2	61.5	59.9	0.60	2,20	11.82	0.54
SAM Fatigue Scale[a]	Scale units	Sham	2.08	2.25	2.42				
		Methanol	2.08	2.79	2.79	2.97	2,20	0.31	0.10
SAM Workload Scale[a]	Scale units	Sham	2.04	2.92	2.83				
		Methanol	2.08	2.79	2.79	0.22	2,20	0.37	0.68
Alertness rating	Scale units	Sham	8.45	7.91	7.73				
		Methanol	8.32	7.64	7.82	0.44	2,18	0.35	0.64
Mood Adjective Check List									
Aggression	Scale scores	Sham	0.25	0.08	0.12				
		Methanol	0.00	0.04	0.08	1.0	2,20	0.17	0.34
Anxiety	Scale scores	Sham	0.54	0.21	0.25				
		Methanol	0.21	0.42	0.21	1.36	2,20	0.65	0.28
Surgency	Scale scores	Sham	1.88	1.88	1.58				
		Methanol	1.58	1.75	1.58	0.34	2,20	0.77	0.64
Elation	Scale scores	Sham	1.71	1.92	1.75				
		Methanol	1.42	1.33	1.38	0.31	2,20	0.87	0.65
Concentration	Scale scores	Sham	3.21	4.62	3.88				
		Methanol	3.46	4.21	4.17	2.84	2,20	0.67	0.09
Fatigue	Scale scores	Sham	1.42	1.67	2.25				
		Methanol	1.08	2.88	2.62	5.34	2,20	1.34	0.02
Social affection	Scale scores	Sham	1.17	1.33	0.83				
		Methanol	1.33	1.00	0.96	2.57	2,20	0.36	0.11
Sadness	Scale scores	Sham	0.04	0.00	0.12				
		Methanol	0.00	0.04	0.04	1.75	2,20	0.03	0.21
Skepticism	Scale scores	Sham	0.08	0.12	0.17				
		Methanol	0.04	0.08	0.08	1.0	2,20	0.01	1.00
Egotism	Scale scores	Sham	0.25	0.33	0.21				
		Methanol	0.17	0.38	0.42	1.03	2,20	0.25	0.35
Vigor	Scale scores	Sham	4.17	4.33	3.38				
		Methanol	4.38	3.46	3.08	2.41	2,20	1.46	0.12

[a] SAM = School of Aerospace Medicine.

Table C.2. Statistics for Dependent Variables Obtained Only in Battery A

Variable	Units	Exposure	Means			F	df	MSE	Corrected p
			Pre	During	Post				
EEG Spectral Analysis									
Mean amplitude									
Delta	μV	Sham	8.22	5.48	5.33				
		Methanol	5.72	6.99	5.33	1.16	2,20	42.77	0.31
Theta	μV	Sham	4.56	3.28	3.23				
		Methanol	3.56	4.34	3.28	0.70	2,20	18.09	0.42
Alpha	μV	Sham	4.10	3.17	3.60				
		Methanol	3.22	3.80	3.57	0.81	2,20	8.42	0.40
Beta	μV	Sham	1.72	1.03	1.21				
		Methanol	1.19	1.56	1.07	1.40	2,20	2.45	0.26
Percent energy									
Delta	Percent	Sham	30.72	30.33	27.59				
		Methanol	29.91	29.97	28.97	0.42	2,20	38.22	0.61
Theta	Percent	Sham	20.04	20.83	18.95				
		Methanol	21.36	21.15	19.75	0.25	2,20	11.58	0.75
Alpha	Percent	Sham	28.94	29.79	33.10				
		Methanol	28.93	28.94	32.42	0.10	2,20	22.61	0.88
Beta	Percent	Sham	20.29	19.05	20.37				
		Methanol	19.81	19.94	18.86	1.17	2,20	14.77	0.33
Peak frequency									
Delta	Cycles/second	Sham	1.08	0.69	0.77				
		Methanol	0.83	0.85	1.08	4.45	2,20	0.23	0.03[a]
Theta	Cycles/second	Sham	5.29	5.58	5.80				
		Methanol	5.71	5.27	5.65	0.59	2,20	3.00	0.56
Alpha	Cycles/second	Sham	9.46	9.00	9.54				
		Methanol	9.06	9.31	9.50	1.09	2,20	1.38	0.33
Beta	Cycles/second	Sham	16.15	16.06	16.10				
		Methanol	16.54	15.60	15.90	0.33	2,20	7.08	0.69
Summary data									
Total amplitude	μV	Sham	96.95	70.24	79.68				
		Methanol	74.98	90.87	76.50	0.92	2,20	5969	0.37
Dominant frequency	Cycles/second	Sham	9.40	8.92	9.34				
		Methanol	8.67	8.69	9.50	1.44	2,20	1.66	0.26
Dominant amplitude	μV	Sham	7.28	6.84	8.06				
		Methanol	5.98	7.58	8.31	0.76	2,20	17.86	0.47
Visual-Event-Related Potential									
Absolute amplitude									
N1	μV	Sham	−1.36	−0.42	0.62				
		Methanol	−1.01	0.01	0.59	0.22	2,20	3.17	0.73
P2	μV	Sham	5.30	5.50	6.44				
		Methanol	5.08	6.14	6.80	1.50	2,20	1.54	0.25
N2	μV	Sham	−1.35	−1.82	−1.30				
		Methanol	−1.30	−1.31	−1.04	0.20	2,20	3.11	0.81
P3	μV	Sham	7.68	6.95	7.73				
		Methanol	7.79	7.75	7.29	0.91	2,20	5.11	0.41
Absolute latency									
N1	msec	Sham	185	182	181				
		Methanol	182	185	182	1.71	2,20	59.7	0.22
P2	msec	Sham	238	239	232				
		Methanol	234	234	233	1.37	2,20	86.9	0.28
N2	msec	Sham	306	308	305				
		Methanol	303	301	309	2.04	2,20	187.2	0.18
P3	msec	Sham	394	395	395				
		Methanol	388	393	389	0.50	2,20	116.4	0.57
Peak-to-trough amplitude									
N1P2	μV	Sham	6.67	5.92	5.83				
		Methanol	6.08	6.13	6.21	1.45	2,20	2.18	0.26
P2N2	μV	Sham	6.66	7.32	7.74				
		Methanol	6.38	7.44	7.83	0.16	2,20	3.64	0.82
N2P3	μV	Sham	9.03	8.76	9.03				
		Methanol	9.09	9.06	8.32	0.90	2,20	3.62	0.42
Peak-to-trough latency									
N1P2	msec	Sham	52.9	56.7	51.7				
		Methanol	52.1	49.6	51.2	1.58	2,20	106.0	0.24
P2N2	msec	Sham	67.9	69.2	72.5				
		Methanol	69.6	66.7	75.4	0.55	2,20	176.5	0.54
N2P3	msec	Sham	88.3	86.7	89.6				
		Methanol	84.6	92.1	80.4	2.50	2,20	260.8	0.13
Auditory-Event-Related Potential									
Absolute amplitude									
N1	μV	Sham	−7.85	−7.91	−7.49				
		Methanol	−7.35	−8.11	−7.59	0.29	2,20	5.80	0.67
P2	μV	Sham	7.22	7.69	7.63				
		Methanol	8.59	8.80	8.77	0.06	2,20	3.87	0.92

(Table continues next page.)

Table C.2. (*continued*)

Variable	Units	Exposure	Means Pre	Means During	Means Post	F	df	MSE	Corrected p
N2	μV	Sham	1.58	1.30	1.24				
		Methanol	1.60	2.36	0.73	1.51	2,20	5.09	0.25
P3	μV	Sham	6.81	6.42	7.21				
		Methanol	7.80	7.92	7.56	0.84	2,20	4.73	0.44
Absolute latency									
N1	msec	Sham	132	137	136				
		Methanol	137	136	136	0.83	2,20	111.9	0.44
P2	msec	Sham	250	244	237				
		Methanol	240	250	240	7.65	2,20	105.3	0.01
N2	msec	Sham	337	335	339				
		Methanol	332	347	334	1.73	2,20	691.5	0.21
P3	msec	Sham	394	399	400				
		Methanol	399	402	405	0.04	2,20	561.9	0.95
Peak-to-trough amplitude									
N1P2	μV	Sham	15.07	15.60	15.12				
		Methanol	15.94	16.91	16.36	0.12	2,20	5.67	0.85
P2N2	μV	Sham	5.65	6.39	6.38				
		Methanol	6.99	6.44	8.04	1.39	2,20	6.32	0.27
N2P3	μV	Sham	5.23	5.12	5.97				
		Methanol	6.20	5.56	6.83	0.20	2,20	4.72	0.75
Peak-to-trough latency									
N1P2	msec	Sham	117	107	101				
		Methanol	103	114	104	5.61	2,20	256.4	0.01
P2N2	msec	Sham	88	91	102				
		Methanol	92	98	94	0.68	2,20	1,028.8	0.50
N2P3	msec	Sham	57	64	62				
		Methanol	68	55	72	3.20	2,20	480.3	0.07
Contingent Negative Variation									
Reaction time	msec	Sham	230	246	232				
		Methanol	232	232	222	0.88	2,18	330.8	0.40
Starting latency	msec	Sham	659	675	637				
		Methanol	620	648	608	0.10	2,18	7,373	0.87
Latency of maximum	msec	Sham	1,741	1,855	1,864				
		Methanol	1,771	1,833	1,884	0.13	2,18	22,040	0.86
Amplitude	μV	Sham	− 16.2	− 16.7	− 19.9				
		Methanol	− 16.2	− 16.4	− 18.7	0.22	2,18	14.8	0.77
Respiration									
Breaths per minute	Breaths/minute	Sham	8.0	8.0	7.7				
		Methanol	7.7	8.5	8.0	0.69	2,18	1.21	0.50
Respiratory amplitude	mV	Sham	55.1	53.7	56.5				
		Methanol	57.0	47.2	55.8	0.76	2,18	119.5	0.45
SD, respiratory amplitude	mV	Sham	6.75	11.49	10.17				
		Methanol	8.61	8.32	7.21	1.06	2,18	38.2	0.36
Cardiac Interbeat Interval									
Mean	msec	Sham	874	968	1,048				
		Methanol	900	948	1,035	2.08	2,20	3,537	0.16
SD	msec	Sham	57.7	77.3	81.9				
		Methanol	62.3	74.2	78.0	0.62	2,20	437.7	0.49
Symbol Digit Substitution Task									
Mean reaction time	Seconds	Sham	1.58	1.68	1.61				
		Methanol	1.67	1.69	1.58	3.17	2,20	0.007	0.07
SD	Seconds	Sham	0.43	0.47	0.46				
		Methanol	0.57	0.53	0.47	1.33	2,20	0.022	0.28
Percent correct	Percent	Sham	98.1	98.3	97.7				
		Methanol	98.7	97.9	98.0	0.27	2,20	4.68	0.74
Three-Choice Reaction Time									
Mean reaction time	Seconds	Sham	0.436	0.425	0.432				
		Methanol	0.436	0.427	0.427	0.31	2,20	0.0002	0.70
SD	Seconds	Sham	0.089	0.094	0.099				
		Methanol	0.093	0.092	0.098	0.20	2,20	0.0003	0.75
Percent correct	Percent	Sham	98.9	98.4	97.9				
		Methanol	98.7	98.4	98.9	1.75	2,20	1.42	0.21
Stroop Color-Word Test									
Number correct, control	Count	Sham	81.4	80.4	82.0				
		Methanol	80.3	82.3	81.8				
Number correct, interference	Count	Sham	63.2	68.1	68.3				
		Methanol	65.6	65.9	67.2	3.59	2,20	12.16	0.05
Number attempted, control	Count	Sham	81.8	81.7	82.8				
		Methanol	81.0	82.3	82.2				
Number attempted, interference	Count	Sham	63.8	68.6	68.8				
		Methanol	66.1	66.5	67.9	2.72	2,20	9.46	0.10
Oddball Task Reaction Time									
Auditory	msec	Sham	378	380	369				
		Methanol	379	362	360	1.51	2,20	359	0.25
Visual	msec	Sham	398	402	391				
		Methanol	394	396	384	0.09	2,20	198	0.89

[a] This apparently significant univariate effect was not interpreted, as no multivariate interaction with $p \leq 0.10$ was observed.

Table C.3. Statistics for Dependent Variables Obtained Only in Battery B

Variable	Units	Exposure	Means			F	df	MSE	Corrected p
			Pre	During	Post				
Visual function									
Depth perception	Angle of stereopsis in seconds of arc	Sham	85.8	91.7	84.2				
		Methanol	82.9	100.4	83.8	0.34	2,20	669	0.60
Near acuity	Snellen equivalents	Sham	17.8	17.9	18.3				
		Methanol	18.4	17.7	18.0	2.34	2,20	0.79	0.12
Far acuity	Snellen equivalents	Sham	22.7	22.4	23.0				
		Methanol	21.8	21.2	21.7	0.18	2,20	1.58	0.81
Far vertical phoria	1/2 Diopters	Sham	0.58	0.75	0.75				
		Methanol	0.75	0.92	0.83	0.08	2,20	0.16	0.83
Far lateral phoria	Diopters	Sham	1.33	0.92	1.25				
		Methanol	1.25	1.25	1.33	0.80	2,20	0.33	0.45
Near vertical phoria	1/2 Diopters	Sham	1.17	1.17	1.00				
		Methanol	1.00	0.92	1.00	0.59	2,20	0.16	0.56
Near lateral phoria	Diopters	Sham	1.75	1.92	2.33				
		Methanol	2.42	1.92	2.08	1.82	2,20	0.74	0.19
Critical flicker fusion frequency	Hz	Sham	36.9	36.3	37.3				
		Methanol	37.2	35.4	35.9	2.23	2,20	2.03	0.15
Hand steadiness	Seconds	Sham	1.72	2.38	1.95				
		Methanol	1.71	2.16	1.79	0.06	2,20	1.03	0.90
Two-letter visual search task									
Reaction time	Seconds	Sham	4.18	4.20	4.02				
		Methanol	4.27	4.11	4.01	0.80	2,20	0.13	0.45
Number correct	Count	Sham	11.50	11.33	11.50				
		Methanol	11.50	11.75	11.71	0.89	2,20	0.58	0.43
Gamberale reaction time									
Reaction time	Seconds	Sham	0.273	0.282	0.279				
		Methanol	0.292	0.318	0.312	0.92	2,20	0.006	0.39
SD	Seconds	Sham	0.051	0.051	0.055				
		Methanol	0.058	0.081	0.086	1.45	2,20	0.008	0.26
Visual tracking task	Root mean squared error	Sham	3.83	3.63	3.80				
		Methanol	3.98	3.77	3.87	0.08	2,20	0.355	0.89
Sternberg memory task									
Reaction time	msec	Sham	635	640	664				
		Methanol	607	654	641	3.57	2,20	7.320	0.05
Slope	msec	Sham	44.0	54.2	66.7				
		Methanol	33.9	44.4	39.3	1.04	2,20	1,183	0.36
Intercept	msec	Sham	462	422	400				
		Methanol	472	478	485	0.74	2,20	23,324	0.46
Interval production task									
Synchrony	Deviation in msec	Sham	- 28.9	- 27.4	- 22.1				
		Methanol	- 25.2	- 27.9	- 10.6	1.10	2,20	410.6	0.34
Task score	Derived score	Sham	6.19	6.47	6.54				
		Methanol	7.58	7.09	7.09	1.55	2,20	1.65	0.24
Speeded addition task									
Number of errors	Count	Sham	2.12	1.58	1.29				
		Methanol	2.42	1.71	1.62	0.16	2,20	0.910	0.81

APPENDIX D. Forms and Questionnaires

ID _____
SESSION _____
DATE _____

Below is a list of problems and complaints that people sometimes have. Read each item carefully, and place an "X" in the box that best describes how much that problem has bothered you in the last 4 hours. Do not skip any items.

	NOT AT ALL	A LITTLE BIT	MODERATELY	QUITE A BIT	EXTREMELY
1. Sweating					
2. Yawning					
3. Trouble getting your breath					
4. Runny eyes					
5. Suddenly scared for no reason					
6. Blurred vision					
7. Feeling low in energy or slowed down					
8. Pains in the heart or chest					
9. Feeling weakness in parts of your body					
10. Eyes stinging or burning					
11. Trouble remembering things					
12. Feeling high in energy, or speeded up					
13. Hot spells					
14. Itching or burning skin					
15. Scratchiness in throat					
16. A lump in your throat					
17. Runny nose					
18. Feeling fearful					
19. Numbness or tingling in parts of your body					
20. Cold spells					
21. Sore throat					
22. Heavy feeling in your arms or legs					

PLEASE TURN QUESTIONNAIRE OVER

Figure D.1. Health effects questionnaire.

	NOT AT ALL	A LITTLE BIT	MODERATELY	QUITE A BIT	EXTREMELY
23. Shaky hands					
24. Faintness or dizziness					
25. Congested nose					
26. Being sleepy too much of the time					
27. Feeling too excitable					
28. Cold hands and feet					
29. Feeling easily annoyed or irritated					
30. Headaches					
31. Heart pounding or racing					
32. Goosebumps					
33. Trouble concentrating					
34. Your mind going blank					
35. Feeling short of air					
36. Feeling no interest in things					
37. Nausea or upset stomach					
38. Feeling tense or keyed up					
39. Feeling so restless you can't sit still					
40. Crawling sensations on your skin					

If you noticed any other problems or complaints during the past four hours, list them here. Then for each one, put an "X" in the box that best describes how much that problem or complaint bothered you.

Figure D.1. Continued.

FULLY ALERT; THINKING EASILY AND QUICKLY AT PEAK EFFICIENCY

10-

1-

FEELING VERY SLOWED DOWN; HARD TO CONCENTRATE OR THINK CLEARLY

Figure D.2. Alertness scale.

NAME | DATE AND TIME

SUBJECTIVE FATIGUE

Circle the number of the statement which describes how you feel RIGHT NOW.

1	Fully Alert; Wide Awake; Extremely Peppy
2	Very Lively; Responsive, But Not At Peak
3	Okay; Somewhat Fresh
4	A Little Tired; Less Than Fresh
5	Moderately Tired; Let Down
6	Extremely Tired; Very Difficult to Concentrate
7	Completely Exhausted; Unable to Function Effectively; Ready to Drop

COMMENTS

WORKLOAD ESTIMATE

Circle the number of the statement which best describes the MAXIMUM workload you experienced during the PAST HOUR.

1	Nothing to do; No Demands
2	Little to do; Minimum Demands
3	Active involvement Required, But Easy to Keep Up
4	Challenging, But Manageable
5	Extremely Busy; Barely Able to Keep Up
6	Too Much to do; Overloaded; Postponing Some Tasks
7	Unmanageable; Unacceptable

COMMENTS

SAM FORM 94 SEP 80 STATUS CHECK

Figure D.3. Status check.

1. In your judgment, were methanol vapors present when you entered the test room?
   ____present
   ____not present
   How confident are you of this judgment? (circle one)

1	2	3	4	5
not at all confident				totally confident

   What are you basing this judgment on?
2. In your judgment, were methanol vapors present just before you left the test room?
   ____present
   ____not present
   How confident are you of this judgment? (circle one)

1	2	3	4	5
not at all confident				totally confident

   What are you basing this judgment on?

Figure D.4. Volunteer's rating form.

1. In your judgment, were methanol vapors present when you entered the test room?
   ____present
   ____not present
   How confident are you of this judgment? (circle one)

1	2	3	4	5
not at all confident				totally confident

   What are you basing this judgment on?

Figure D.5. Experimenter's rating form.

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ABBREVIATIONS

ACGIH	American Conference of Government and Industrial Hygienists
A/D	analog-to-digital
DC	direct current
df	degrees of freedom
ECG	electrocardiogram
EEG	electroencephalogram
EOG	electrooculogram
EPA	Environmental Protection Agency
F	F ratio in analysis of variance
FFT	Fast Fourier Transform
MRI	Midwest Research Institute
MSE	mean-squared error
NAD	nicotinamide adenine dinucleotide (oxidized form)
NIOSH	National Institute of Occupational Safety and Health
ppm	parts per million
R^2	multiple correlation coefficient
SD	standard deviation
WHO	World Health Organization

INTRODUCTION

A Request for Applications (RFA 87–2), soliciting proposals for studies of "Behavioral and Neurotoxicological Effects of Methanol and Other Components of Automotive Emissions," was issued by the Health Effects Institute (HEI) in the summer of 1987. In response to the RFA, Dr. M. R. Cook, from the Midwest Research Institute, submitted a proposal entitled "Effects of Methanol Vapor on Human Function." The HEI approved an 18-month project for a pilot study that began in July 1988. Total expenditures were $375,693. The Investigators' Report was received at the HEI in March 1990 and was accepted by the Health Review Committee in October 1990. During the review of the Investigators' Report, the Review Committee and the principal investigator had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the Review Committee's Commentary. The Health Review Committee's Commentary is intended to place the Investigators' Report in perspective as an aid to the sponsors of the HEI and to the public.

REGULATORY BACKGROUND

The U.S. Environmental Protection Agency (EPA) sets emissions standards for diesel engines and vehicles under Section 202 of the Clean Air Act, as amended in 1990. Section 202(a)(1) of the Act directs the Administrator of the EPA to "prescribe (and from time to time revise) . . . standards applicable to the emissions of any air pollutant from any class or classes of new motor vehicles or new motor vehicle engines, which in his judgment cause, or contribute to, air pollution which may reasonably be anticipated to endanger public health or welfare."

Under the authority granted it by Section 202(a)(1), the EPA issued exhaust emissions standards in 1989 effective for all 1990 model year light-duty cars, light-duty trucks, heavy-duty trucks, and motorcycles using methanol fuel. The methanol emission standards are comparable to the emission standards applicable to both gasoline- and diesel-powered engines. New evaporative emissions standards applicable to light-duty vehicles, light-duty trucks, and heavy-duty trucks will soon be finalized by the EPA.

The Clean Air Act Amendments of 1990 instituted a number of changes in existing laws that relate to methanol. Although described as a "hazardous air pollutant" under Section 112(a)(6) and (b) of the Act, as amended by Section 301 of the 1990 Amendments, methanol is also defined both as a "low polluting fuel" under Section 219(f)(2) of the Act, as added by Section 227 of the Amendments, and as a "clean alternative fuel" under Section 241(2) of the Act, as added by Section 229 of the Amendments. Under Section 219(c)(2) as added by Section 227 of the Amendments, the use of low-polluting fuels would be required for all "urban buses" used in "all metropolitan statistical areas or consolidated metropolitan statistical areas with a 1980 population of 750,000 or more" if certain particulate matter targets are not met. In addition, the Administrator may extend the use of low-polluting fuels for urban buses in areas where the 1980 population was less than 750,000 if "a significant benefit to public health could be expected to result from such extension."

Sections 241 through 250 of the Act, as added by Section 229 of the Amendments, also requires the Administrator to implement a program for "clean-fuel vehicles" to be used by some "centrally fueled fleets" in various nonattainment areas. Although a "clean-fuel" vehicle is defined by Section 241(7) in terms of vehicle performance rather than fuel composition, methanol is specifically listed in Section 241(2) as a possible "clean alternative fuel." A pilot "clean-fuel vehicle" program for California is also mandated by Section 249 of the Act, as amended in 1990.

SCIENTIFIC BACKGROUND

The continued inability of many urban locales to meet air quality standards is due, in large part, to the atmospheric ozone produced when hydrocarbons are released through evaporative emissions, exhaust emissions, and refueling operations associated with the use of gasoline-powered vehicles. The use of methanol as an alternative motor vehicle fuel may offer important advantages to human health and quality of life by decreasing hydrocarbon emissions and thereby potentially reducing atmospheric ozone concentrations. Although the substitution of methanol for gasoline promises to be an important remedial action, it raises substantive concerns about potential health risks posed by increased exposure of the general population to methanol vapors.

In a Special Report issued in 1987, the Health Effects Institute evaluated the existing information on the biological effects of methanol to determine what health problems, if any, might emerge if methanol were to become more widely used as an automotive fuel (reviewed by Health Effects Institute 1987). Using exposure assessments available at that time, the HEI concluded that brief and intermittent exposure to methanol vapor from evaporative emissions or exhaust emissions from methanol-fueled vehicles probably

would not result in adverse health effects. However, the HEI recognized that there had not been sufficient research to eliminate the possibility that health effects could occur in human subjects chronically exposed to low levels of methanol.

The following questions, which are relevant to a discussion of the possible subtle effects on human health from exposures to low concentrations of methanol vapor, will be addressed subsequently: (1) What are the acute toxic effects of high doses of methanol to humans? (2) How is methanol metabolized in humans and how does metabolism relate to toxicity? (3) What concentrations of methanol vapors have humans been exposed to occupationally, and how do they compare to the environmental exposure concentrations anticipated should methanol-fueled vehicles come into widespread use? and, finally, (4) Given the documented neurotoxicity of large doses of ingested methanol to human motor function and the visual system, what neurotoxicological tests are appropriate for measuring possible subtle effects on the central nervous system resulting from inhalation of low concentrations of methanol vapors?

TOXICITY OF METHANOL

The acute toxicity of methanol (wood alcohol) to humans has been recognized for some time, with numerous episodes of poisoning documented since the late nineteenth century (Posner 1975; reviewed by Kavet and Nauss 1990). These poisonings usually have stemmed from accidental or intentional ingestion of liquids containing methanol, but in some cases, inhalation or dermal exposure has been implicated in workplace settings (Posner 1975). Although individual susceptibility to methanol poisoning varies, a dose of 300 mg/kg of body weight (approximately one-quarter of a cup for an adult) generally is regarded as the lowest single lethal dose (Kavet and Nauss 1990).

Classic symptoms of acute methanol poisoning include a short period of mild intoxication, followed by a variable asymptomatic period of 12 to 24 hours, and ending with the development of visual disturbances (eye pain, blurred vision, constriction of visual fields) that may progress to temporary or permanent blindness, coma, and death from respiratory failure (Tephly and McMartin 1984; Kavet and Nauss 1990; reviewed by Tephly 1991). Concurrent with the onset of visual symptoms, there is also a marked metabolic acidosis (increased blood acidity and a concomitant loss in blood-buffering capacity).

The neurotoxic effects of methanol on the visual system involve optic disc edema and consequent swelling of the axons in the optic disc and the adjacent portions of the optic nerve (Kavet and Nauss 1990). In addition, permanent motor impairment consisting of tremors (spasticity) and limited and painful movement of the limbs (hypokinetic dystonia) has been reported in individuals ingesting near-fatal amounts of methanol (Guggenheim et al. 1971; Ley and Gali 1983; LeWitt and Martin 1988). Furthermore, a computed axial tomography (CAT) scan of a patient with permanent motor impairment from methanol intoxication showed brain lesions of the putamen that appear to be consistent with the necrosis of the putamen often observed in autopsies of patients who have died of acute methanol poisoning (LeWitt and Martin 1988).

Because of these neurotoxic effects, it is appropriate to examine the premise that exposure to low concentrations of methanol vapor for sufficient periods may induce small, but significant, disturbances in central nervous system function. There is, however, little available information on this topic. Two early accounts in Soviet literature from different laboratories reported that exposure of humans by inhalation to very low concentrations of methanol (less than 12 mg/m^3) stimulated visual and olfactory receptors (Health Effects Institute 1987; Kavet and Nauss 1990). One of the studies reported an EEG threshold for conditioned reflexes at only 1.17 mg of methanol/m^3. Both of these studies were deficient in several respects: There was no description of the subjects, several important details of the experimental procedure were not given, and there was no information provided on methanol purity and the analytical techniques used for determining methanol concentration.

In a study of teachers' aides who complained of physical ailments when exposed to vapors from direct-process duplicators, methanol concentrations ranged from 365 to 3,080 ppm (mean concentration of 1,060 ppm) in the poorly ventilated rooms containing the machines (Frederick et al. 1984). Symptoms known to be associated with methanol toxicity (headache, dizziness, blurred vision, and nausea) were reported significantly more often by aides than by a relatively nonexposed group of teachers. The incidence of symptoms was correlated with the amount of time the aides spent near the duplicators. This study, however, was conducted on the basis of the teachers' aides' complaints, and thus may have been compromised by responder bias. Also, the complaints were based on symptoms without accompanying clinical observations. Finally, the possibility that other chemicals or solvents were responsible for the aides' complaints was not excluded (Kavet and Nauss 1990).

METHANOL METABOLISM

Because methanol is rapidly and uniformly distributed to body tissues in proportion to their water content, the route of exposure is not important in determining body burden (Tephly and McMartin 1984). Methanol is rapidly cleared from the body either by direct excretion in urine and ex-

haled air, or by metabolism to carbon dioxide. The half-life of methanol in the blood and urine is approximately three hours after either oral ingestion or inhalation exposure (Kavet and Nauss 1990; Tephly 1991).

Methanol is metabolized first to formaldehyde, then to formate (the putative toxic metabolite of methanol), and finally to carbon dioxide and water (Tephly and McMartin 1984; Tephly 1991). Although this pathway is common to all mammalian species studied, rats metabolize formate to carbon dioxide more efficiently than humans and other primates. Thus, blood formate levels do not reach toxic levels in rats. This fact explains why attempts to duplicate the ocular toxicity and metabolic acidosis associated with methanol toxicity in humans have not been successful in rodents. Monkeys, however, are good models for acute methanol poisoning in humans. Single doses of methanol (3 to 4 g/kg) induced severe metabolic acidosis in monkeys, which was accompanied by a sharp increase in blood formate (Clay et al. 1975; McMartin et al. 1975). Optical lesions (optic disc edema from axonal swelling) also developed with doses of methanol sufficient to induce metabolic acidosis (Baumbach et al. 1977; Hayreh et al. 1977; Martin-Amat et al. 1977). The primary role of formate, rather than the condition of acidosis itself, in the development of optic disc edema was established by experiments in which buffered formate was infused into monkeys to prevent the acidosis-associated drop in pH (Martin-Amat et al. 1978).

HUMAN EXPOSURE TO METHANOL

Individuals are exposed to methanol in many workplace environments. The current threshold limit value for methanol for an eight-hour day and a 40-hour workweek is a time-weighted average of 200 ppm (261 mg/m^3) (American Conference of Governmental Industrial Hygienists 1990). The National Institute for Occupational Safety and Health recommendation includes this standard with a further limitation of a ceiling concentration of 800 ppm over any 15-minute period of the working day (National Institute of Governmental Industrial Hygienists 1976).

It is expected that the most widespread human exposure to methanol would be via inhalation of vapors from methanol-fueled vehicles. Worst-case exposure concentrations for methanol vapor are estimated by the EPA to vary from less than 0.1 to approximately 250–500 mg of methanol/m^3 for "hot soak" conditions (evaporative emissions from hot engines after being turned off) in personal garages with malfunctioning vehicles (Gold and Moulis 1988; Kavet and Nauss 1990). Exposure times in this scenario are expected to be less than 15 minutes (Kavet and Nauss 1990). Refueling of vehicles could result in a three- to four-minute exposure to methanol at 30 to 50 mg/m^3 (Harvey et al. 1984). Much higher individual exposure could be expected occasionally from accidental or intentional ingestion, inhalation, or dermal absorption of methanol fuel.

It is clear that significant increases in blood and urine methanol and formate levels can be measured in humans exposed to methanol vapors in the workplace at concentrations below the threshold limit value of 200 ppm. Printing office and chemical workers exposed to approximately 100 ppm methanol during the workshift exhibited a 1.5- to 2.5-fold increase in blood and urinary formate and a 15- to 20-fold increase in blood and urinary methanol at the end of the working day, whereas nonexposed workers did not exhibit increases (Baumann and Angerer 1979; Heinrich and Angerer 1982). In a different study of methanol-exposed workers, a 16-hour postexposure period was required to account for all of the formate produced and to obtain a linear correlation between methanol concentration in air and urinary formate excretion (Liesivuori and Savolainen 1987). These data indicate that sufficient time will not have elapsed for measurement of blood or urine formate if samples are taken immediately after short-term exposure to low concentrations of methanol.

It is important to note that humans are also routinely exposed to methanol from both diet and the metabolic breakdown of certain amino acids (Kavet and Nauss 1990). Principal dietary sources of methanol include fruit and vegetable products, fermented beverages, and foods and beverages artificially sweetened with aspartame, a methyl ester dipeptide that is hydrolyzed to methanol and amino acids during digestion. Dietary intake provides much of the methanol and formate background values in blood and urine, and is a useful marker by which to judge the magnitude of other types of methanol exposure.

The concept of body burden, based on the calculated amount of methanol initially present in the body, can also be used to compare the impact of different methanol exposure scenarios. For example, estimated body burdens for the EPA worst-case conditions of methanol exposure range from 0.0004 mg/kg of body weight for one hour of expressway exposure to 0.6 mg/kg for the 15-minute personal garage "hot soak" scenario (Health Effects Institute 1989). By comparison, consumption of one 12-oz. soft drink sweetened with aspartame would result in a body burden of 0.28 mg/kg, or about one-half of the highest body burden in the scenarios described above.

NEUROTOXICOLOGICAL TESTING IN HUMANS

Detailed research using behavioral tests with human subjects to evaluate chemical effects on the nervous system began when Beard and Wertheim (1968) identified behavioral changes (reduced ability to estimate stimulus duration) in

human volunteers after low exposures (50 ppm) to carbon monoxide. This finding was used to establish the first federal standards that were based on a reversible, apparently pharmacologic, central nervous system effect of uncertain relevance to health. Attempts to replicate the finding were generally not successful (Laties and Merigan 1979), but the research spawned by these results has contributed significantly to the formulation of subsequent standards for human exposure levels to environmental chemicals. During the 1970s, sophisticated, quantitative behavioral and electrophysiological tests of peripheral and central nervous system function began to appear in toxicologic assessments with increasing frequency (Xintaras et al. 1974; Weiss and Laties 1975). This trend continues to accelerate in the laboratory testing of human subjects (Dick and Johnson 1986; U.S. Congress 1990), particularly individuals occupationally exposed to solvents (Anger 1990).

The extent of nervous system changes produced by chemicals can only be estimated. Anger and Johnson (1985) identified approximately 750 chemicals that, directly or indirectly, altered nervous system function. They identified 120 different effects related to exposure to industrial chemicals, including those associated with the motor, sensory, affective, and cognitive aspects of human nervous system function. The large diversity in potential nervous system effects is a major factor complicating the selection of tests for an experimental study.

There are a number of approaches to selecting tests. Theoretically, the best approach is to employ a battery of tests that thoroughly characterizes the full range of nervous system functions. In practice, this approach is never used because of the time and financial limitations inherent in any test setting and the difficulty of obtaining agreement on the full range of functions to be measured. Thus, no single neurotoxicologic study can fully characterize the entire range of potential neurotoxic effects.

A second approach to selecting tests is to employ only those tests that evaluate the effects that occur most frequently after exposure to toxic chemicals. This approach, however, ignores those functions that are measured only infrequently (for example, smell, balance, vigilance, certain kinds of memory, intelligence, and personality). Therefore, this approach could be inappropriate for a chemical for which no prior information is available.

When there is some knowledge of the effects of the chemical under study, a third approach is preferable. In this case, tests can be selected based on a knowledge of the established effects of the chemical. A major limitation in this approach is that insufficient information exists for most chemicals, so no certain selection of appropriate tests is possible. However, there is considerable information on the effects of methanol on the visual system; thus, the battery of tests could be weighted to include those that rely, in large measure, on visual acuity.

In general, an acceptable approach is to focus on a reasonable selection of tests to monitor a range of nervous system functions, taking into account any known effects of the chemical in question, or its structural analogues. It would be valuable to include in the battery several tests that repeatedly have demonstrated general sensitivity to chemical effects.

In summary, the projected increased use of methanol as an alternative motor vehicle fuel will result in increased exposure of the general population to methanol vapor. Given the well-documented disturbances in visual function and the impairment of motor coordination attributed to ingestion of high doses of methanol, it is important to understand the possible consequences to human health of inhalation exposure to relatively low, environmentally relevant concentrations of methanol vapor. There is a critical lack of information about possibly subtle, yet significant neurotoxicological effects of methanol exposure. These effects could have a range of implications with respect to human safety and quality of life.

JUSTIFICATION FOR THE STUDY

In 1987, the HEI sought proposals to investigate the neurotoxicological effects of components of automotive emissions. Because of its potential for increased use as an automotive fuel, special emphasis was placed on methanol. Studies of behavioral changes were stressed because these endpoints could turn out to be early and sensitive indicators of adverse effects of methanol, particularly in view of the documented acute effects of methanol ingestion on motor coordination and visual system function. Subtle and transient behavioral effects, which may fail to be reflected in morphological or physiological measurements, could be significant if performance or quality of life is affected.

Research proposals were encouraged to address five specific objectives: (1) to measure behavioral effects in animals; (2) to evaluate behavioral and cognitive function in humans; (3) to evaluate effects on quality of life and their significance in humans; (4) to measure effects on human nervous system function (particularly vision) with acute and chronic exposure to low and moderate levels of methanol; and (5) to measure human blood methanol and formate levels after exposure to low concentrations of methanol. Dr. Cook's proposal addressed four of the five specific research objectives initially suggested by the HEI. A major strength of the proposal was the fact that information on central nervous system function would be obtained from human subjects exposed to environmentally relevant levels of methanol.

The investigator proposed to evaluate the neurotoxicological effects of inhaled methanol vapors on a variety of behavioral, cognitive, visual, and physiological functions in selected human volunteers under controlled double-blind conditions. Urinary and blood measurements of methanol and formate were planned to assess individual differences in body burdens of methanol.

This project was designed as a preliminary study to determine whether short-term exposure to methanol, at a concentration near the threshold limit value, would lead to measurable deficits in neurobehavioral and neurophysiological functions. The Research Committee believed that the results from this pilot study would serve as a rational basis to guide future research planning, before committing financial resources to a full-scale definitive study. Informed decisions would be made in selecting future dose levels and exposure periods, as well as in the types of tests and measurements that would most certainly detect any consistent, significant effects. Such data would have obvious and important implications for assessing risks to human health from environmental exposure to methanol and would provide further insight into the metabolism and mechanism of action of methanol on the human central nervous system.

OBJECTIVES AND STUDY DESIGN

The overall objective of Dr. Cook's proposal was to carry out a pilot study to evaluate the neurotoxicological effects of low concentrations of inhaled methanol vapors on a variety of behavioral, cognitive, visual, and physiological functions in selected human volunteers under rigorously controlled double-blind exposure conditions.

The study population consisted of 12 healthy male subjects exposed to methanol vapor at 250 mg/m^3 (192 ppm) for 75 minutes. The individuals served as their own controls and participated in two methanol and two sham (filtered air) experimental sessions. Each subject's performance on a battery of neurobehavioral and neurophysiological tests was assessed before, during, and after exposure. Because the tests were too extensive to administer in a single experimental session, they were divided into two parts (Batteries A and B). Battery A consisted of subjective, physiological, and simple cognitive tasks; Battery B measured visual and psychomotor function, more complex cognitive functions, and dual tasks. Subjects were separated randomly into two groups, one of which first was trained and tested on Battery A and one which first was trained and tested on Battery B. Half of the subjects in each group were selected randomly and underwent exposures in the order of sham-methanol-sham-methanol; the other half of each group underwent exposures in the order of methanol-sham-methanol-sham.

To assess the success of the double-blind design, questionnaires were administered to the subjects and to the experimenter who collected the neurobehavioral data after each exposure. Respondents were asked to judge whether they believed that methanol had been present.

Measurements of methanol and formate levels in urine and blood were taken before and immediately after each exposure to correlate possible changes in neurobehavioral measurements with individual body burdens. A third urine sample was taken at the end of the experimental session, approximately one hour after the end of exposure.

TECHNICAL EVALUATION

ATTAINMENT OF STUDY OBJECTIVES

The investigators successfully conducted a careful pilot study that was intended to guide the design and implementation of future studies. The experiments employed well-chosen behavioral tests to evaluate the neurotoxicological effects of acute exposure to methanol vapors in an experimental chamber.

The lack of previous investigations of the neurobehavioral effects of methanol in humans justified the broad approach and selection of many possibly relevant tests, rather than the formulation and testing of specific hypotheses.

METHODS AND STUDY DESIGN

The investigators chose a conservative and appropriate approach to test selection. Tests included those that assess vision, motor performance, and a variety of cognitive domains. The cognitive tests (symbol-digit substitution, Stroop color-word, critical flicker fusion frequency, Sternberg memory, interval production, two-letter visual search, three-choice reaction time, Gamberale reaction time, speeded addition) have been used extensively in laboratory and worksite studies of acute and chronic exposures to chemicals, including ethanol; the tests of vision (depth, color, peripheral vision, binocular acuity) and motor performance (steadiness, tracking, elements of reaction time) also are widely accepted for this purpose (Dick and Johnson 1986; Anger 1990).

The investigators also made special efforts to assess the quality of their double-blind design, an important and often neglected consideration.

STATISTICAL METHODS

The statistical analysis of the study was appropriate and

clearly presented. The data were analyzed with a repeated measures analysis of variance, with variables including exposure category (sham or methanol), exposure order (sham-methanol-sham-methanol or methanol-sham-methanol-sham), and testing period (results from tests administered before, during, and after exposure). Treatment effects were tested by the exposure-by-testing period interaction because this provided the clearest evidence about whether or not methanol exposure had affected the outcome of the test. Several statistically significant treatment-by-testing period interactions were observed in the study.

A preliminary correlation analysis identified clusters of outcome variables with pairwise correlations that were statistically significant at the 0.01 level. These clusters were analyzed together by multivariate repeated measures analysis of variance, which reduced the number of primary tests of the null hypothesis, no methanol effect, from 109 to 30. The method of developing clusters is an appropriate one and is a good strategy for focusing the analysis on more generic questions. However, using a significance level of 0.05 for each of 30 independent tests of significance implies that 1.5 false-positive results would be expected if all null hypotheses were true. Variables that were not correlated with other variables were analyzed by univariate repeated measures analysis of variance.

The investigators reported that only 15 of the 4,152 planned measurements (0.36 percent) were missed. Because the data were so nearly complete and because the missing values were not concentrated in a single variable, the remedial measures used in this study to estimate missing data points were appropriate.

RESULTS AND INTERPRETATION

Exposure Conditions

The measured mean methanol concentrations were uniformly close to the target level of 250 mg/m^3. Some variation in humidity, barometric pressure, and carbon monoxide concentrations between exposure conditions and experimental sessions was noted, but this variation appears to have been too small to affect test results.

Analysis of data from the questionnaires assessing the double-blind design of the study indicated that subjects were not able to judge the presence or absence of methanol at better-than-chance levels. However, there is some evidence suggesting that this design may have been compromised by the faint smell of methanol because the experimenter correctly identified 69 percent of the exposure conditions.

Neurobehavioral Measurements

The study included a large number of neurobehavioral and neurophysiological endpoints, most of which showed no association with methanol exposure. A small number of statistically significant, exposure-related effects and trends were found, but these were not confined to a specific group of tests. They included subjective ratings of fatigue, and certain differences in the timing of peaks (latency) in the complex electrical waveforms evoked in the brain by both visual and auditory stimuli (increased latency of the P200 peak and increased peak-to-trough latency between the N100 and P200 peaks).

Marginally significant effects were found for subjective levels of concentration and performance in the Sternberg memory task. Although statistically significant, the average values for the subjects exposed to methanol were not outside the range of values observed with the subjects in sham exposures.

Subjects also reported increased fatigue during methanol exposure. However, the strongest independent variable predicting the reporting of fatigue was the number of correct judgments of exposure made by the subject during the assessment of the integrity of the double-blind study design. Thus, the subject's impression of fatigue may have been an anticipated effect of his perception of exposure to methanol.

The statistically significant exposure-by-testing period interactions reported in this study are not necessarily related to methanol exposure. In many cases, the differences arose because the base-line preexposure values from the subjects to be exposed to methanol were lower than the corresponding values from the sham-exposure group. This was due presumably to random variability of performance. However, the differences in the latency of the P200 peak from the visual event-related potential depended on the order of exposure and had no obvious interpretation. Neither slope nor intercept on the Sternberg memory task was significantly affected by exposure. However, the multivariate analysis of variance was statistically significant.

The large number of tests performed and the alternative explanations available for each of the positive results do not reflect negatively on the design or analysis of this pilot study. They do raise the possibility, however, that none of these effects will be reproducible. This is supported by the lack of consistency in the small changes measured among related tests in this study. For example, it would be expected that the reported reduction in concentration and the increased subjective feelings of fatigue would affect performance on all of the neurobehavioral tests. Also, a reduction in the reaction time on the Sternberg memory task should have been complemented with a slower reaction time in the Symbol Digit substitution task. Several other electrophysio-

logical measures of the auditory and visual event–related potentials also might have been expected to change in the same way that the P200 components changed. Given the plausible role of chance in obtaining positive results in this study and the uncertain significance of changes in the event-related potentials, it is inappropriate to conclude that these positive effects are attributed to methanol exposure.

Chemical Analyses

Blood and urinary methanol and blood formate measurements were performed on samples taken before and immediately after the 75-minute exposure to methanol vapors. A third urine sample was taken approximately one hour after the end of the experimental session. Methanol concentrations in the urine and blood of methanol-exposed subjects significantly increased by 2.5- and 3.3-fold, respectively, immediately after exposure. Urinary methanol concentrations remained elevated one hour after exposure.

No significant changes were noted in plasma levels of formate, the putative toxic metabolite of methanol (Martin-Amat et al. 1978). The investigators commented correctly that no increase in plasma formate would be detected in the short period (approximately 75 minutes) elapsing between the start of exposure to methanol and the time the blood sample was taken.

IMPLICATIONS FOR FUTURE RESEARCH

Additional research is needed to determine whether exposure to methanol vapor, at concentrations and durations likely to be experienced with increased future use of methanol as an alternative fuel, will have deleterious effects on occupational groups or the general population. A larger number of subjects needs to be tested, before and after exposure to a range of expected ambient methanol vapor concentrations over longer time periods, to determine if there are consistent, dose-related neurotoxicological effects. Subjects should also be selected from potentially susceptible populations, including elderly men and women, and individuals with visual impairments.

Although no substantial changes in performance were detected for many tasks in the pilot study, it cannot be assumed that no changes would occur in studies with other segments of the population. It is, therefore, inappropriate to eliminate any specific tests from a definitive study based on negative effects in the present pilot study. The selection of tests for a definitive study should include clusters of related tasks so that consistent, biologically plausible effects can be distinguished from chance findings that are to be expected when many tests are performed.

To improve reproducibility and sensitivity, all aspects of the testing procedures should be standardized and intersubject and intrasubject variability should be minimized by training the subjects and the tester. More complex tasks (for example, tests of memory with varied levels of difficulty) and more adverse conditions, such as variable illumination in tests of visual system function, should be used. Interaction between the tester and the subjects should be reduced as much as possible, and if several testers are used, observer variability should be measured at the outset and monitored throughout the study. Standards for certification of testers should be developed, and recertification at appropriate intervals should be dependent on documented satisfactory performance.

Because formate has been implicated as the metabolite responsible for the visual toxicity of methanol (Tephly and McMartin 1984), there is concern that sufficient time did not elapse after methanol exposure to allow plasma formate concentrations to reach levels at which they might affect visual system function as assessed by neurotoxicological tests. There is evidence in monkeys and humans that a period of 12 to 16 hours is needed after exposure to methanol before plasma formate reaches maximum levels (McMartin et al. 1975; Liesivuori and Savolainen 1987).

Regardless of when blood samples were taken for analysis, there is a question of whether the total body burden of methanol from the inhalation exposure in this study would have resulted in a detectable elevation of blood formate. There is evidence, however, that blood formate concentrations are elevated substantially during daily occupational exposure to lower doses of methanol for longer periods (40 to 160 ppm for eight hours per day, five days per week) than the exposure conditions used in this study (Baumann and Angerer 1979; Liesivuori and Savolainen 1987). Further information on the pharmacokinetics of methanol metabolism in human subjects is needed to determine the optimal timing of neurobehavioral tests to coincide with maximum concentrations of serum formate.

Exposure of a group of subjects to a positive control (for example, ethanol by ingestion) is recommended to assess impairment in test performance and to validate the consistency of the patterns of results. In addition, blood ethanol should be assayed in test subjects before exposure to methanol or filtered air to rule out confounding effects from the ethanol itself or from its inhibition of methanol metabolism. Also, prescription and nonprescription drug use by potential subjects should be ascertained because certain medications may interfere with the pharmacokinetics and

metabolism of methanol. This problem would be particularly prevalent among older subjects.

CONCLUSIONS

The investigators conducted a carefully designed double-blind pilot study to evaluate the neurotoxicological effects of inhaled methanol vapors in 12 healthy young male subjects. A relatively high methanol vapor concentration of 250 mg/m^3 (192 ppm) was employed in 75-minute exposures. Each subject served as his own control in two methanol and two sham (filtered air) experimental sessions. A total of over 20 appropriately selected neurobehavioral and neurophysiological tests, measuring a variety of visual, behavioral, cognitive, and physiological functions, were administered before, during, and after exposure. These tests were divided into two separate test batteries for each of the two methanol or sham experimental sessions. Urine and blood samples for methanol and formate analysis were taken before and immediately after each exposure as an independent measure of methanol body burden and metabolism to formate, its putative toxic metabolite.

No effects were observed in most of the tests. However, a small number of statistically significant exposure-associated effects were noted in subjective ratings of fatigue and in measurements of time differences in the appearance of certain peaks of electrical activity in the brain evoked by visual and auditory stimuli (visual and auditory event-related potentials). Marginally significant effects were found in subjective ratings of concentration and in measurements of performance in the Sternberg memory task.

Although significant differences were found, the average performance on these tests by subjects exposed to methanol was not outside the range of test values for subjects in sham exposures. Furthermore, in most cases, the differences in test performances between exposed and nonexposed subjects were due primarily to increased base-line preexposure values in the control subjects, which presumably resulted from random variability of performance. The possibility that some of these positive results were due to chance or normal variability is supported by the lack of similar findings in related tests. For example, significant responses in some tests were not complemented by equivalent significant responses in related tests. The design and thoughtful analysis of this pilot study made important contributions in revealing these small inconsistencies.

Methanol concentrations in blood and urine increased by approximately threefold at the end of the experimental sessions. As expected, no increases in blood formate were detected because insufficient time had elapsed for significant metabolism of methanol. Because formate has been implicated as the metabolite of methanol responsible for visual toxicity in monkeys, the question remains whether more significant findings would have been obtained if the subjects had been tested at later times.

These preliminary results are sufficient to warrant a more definitive study in which larger numbers of subjects, representing potentially susceptible populations, would be exposed to methanol concentrations and durations likely to be experienced with the use of methanol-fueled vehicles. In particular, older individuals of both genders and individuals with visual impairments should be tested, with appropriate measures taken to safeguard health during and after exposures.

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Special Reports

Title	Publication Date
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research	September 1985
Automotive Methanol Vapors and Human Health: An Evaluation of Existing Scientific Information and Issues for Future Research	May 1987
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research (Supplement)	January 1988

Research Reports

Report No.	Title	Principal Investigator	Publication Date
1	Estimation of Risk of Glucose 6-Phosphate Dehydrogenase-Deficient Red Cells to Ozone and Nitrogen Dioxide	M. Amoruso	August 1985
2	Disposition and Metabolism of Free and Particle-Associated Nitropyrenes After Inhalation	J. Bond	February 1986
3	Transport of Macromolecules and Particles at Target Sites for Deposition of Air Pollutants	T. Crocker	February 1986
4	The Metabolic Activation and DNA Adducts of Dinitropyrenes	F.A. Beland	August 1986
5	An Investigation into the Effect of a Ceramic Particle Trap on the Chemical Mutagens in Diesel Exhaust	S.T. Bagley	January 1987
6	Effect of Nitrogen Dioxide, Ozone, and Peroxyacetyl Nitrate on Metabolic and Pulmonary Function	D.M. Drechsler-Parks	April 1987
7	DNA Adducts of Nitropyrene Detected by Specific Antibodies	J.D. Groopman	April 1987
8	Effects of Inhaled Nitrogen Dioxide and Diesel Exhaust on Developing Lung	J.L. Mauderly	May 1987
9	Biochemical and Metabolic Response to Nitrogen Dioxide-Induced Endothelial Injury	J.M. Patel	June 1987
10	Predictive Models for Deposition of Inhaled Diesel Exhaust Particles in Humans and Laboratory Species	C.P. Yu	July 1987
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19	Factors Affecting Possible Carcinogenicity of Inhaled Nitropyrene Aerosols	R.K. Wolff	August 1988
20	Modulation of Pulmonary Defense Mechanisms Against Viral and Bacterial Infections by Acute Exposures to Nitrogen Dioxide	G.J. Jakab	October 1988
21	Maximal Aerobic Capacity at Several Ambient Concentrations of Carbon Monoxide at Several Altitudes	S.M. Horvath	December 1988
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29	Early Markers of Lung Injury	J.N. Evans	September 1989
30	Influence of Experimental Pulmonary Emphysema on Toxicological Effects from Inhaled Nitrogen Dioxide and Diesel Exhaust	J.L. Mauderly	October 1989
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The Institute is structured to define, select, support, and review research that is aimed at investigating the possible health effects of mobile source emissions. Its research program is developed by the Health Research Committee, a multidisciplinary group of scientists knowledgeable about the complex problems involved in determining the health effects of mobile source emissions. The Committee seeks advice from HEI's sponsors and from other sources prior to independently determining the research priorities of the Institute.

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