APPENDIX I

EXPOSURE SCENARIOS, OFFSETS, AND SOURCES OF EXPOSURE DATA IN TABLES 3 THROUGH 5

The material in this appendix amplifies the discussions in Section II that describe the exposure levels of methanol that EPA analyses project will occur in different exposure scenarios. The following sections (1) describe the specific exposure scenarios that have been modeled, (2) discuss offsets as they relate to the data presented in Tables 3 through 5 of the report, and (3) identify the sources of data found in Tables 3 through 5.

(1) SCENARIOS

Street Canyon

Typical: four-lane canyon with a traffic load of 800 vehicles per hour; exposure on adjacent sidewalk

Severe: six-lane canyon with a traffic load of 2,400 vehicles per hour; exposure on adjacent sidewalk

Roadway Tunnel

Typical: 1,000 to 2,000 foot tunnel, with average daily traffic of between 10,000 and 15,000 vehicles per day, with longitudinal or semi-transverse ventilation at a rate of approximately 0.17 m^3/sec per lane-meter; exposure in vehicle

Severe: tunnel over 5,000 feet long with average daily traffic in excess of 15,000 vehicles per day, transversely ventilated with a ventilation rate less than 0.11 m^3/sec per lane-meter; exposure in vehicle

Expressway

Typical: a four-lane roadway with a traffic load 1,400 vehicles per hour and a 1 m/sec wind 315 degrees to the direction of travel; exposure in the vehicle

Severe: a ten-lane roadway with a traffic load of 3,675 vehicles per hour; same wind conditions; exposure in vehicle

Off-Road: exposure 100 meters downwind of an eight-lane highway carrying 15,000 vehicles per hour (e.g., morning rush hour); wind perpendicular to road

Parking Garage

Trip Start (Idle)

Typical: above ground naturally ventilated garage

Severe: underground garage; exposure is at lowest parking level 20 minutes after the garage is emptying from a full condition

Hot-soak

Typical: above ground, naturally ventilated garage; all vehicles in a full garage within the first hour of hot-soak

Severe: same as typical except underground

Personal Garage

Trip Start (Idle)

Typical: garage door raised; 30-second warm-up; natural ventilation

Severe: same as above with 5-minute warm-up

Hot-soak

Typical: garage door closed; ventilation rate of 20 cubic feet per minute for typical sized garage (62 m^3)

Severe: door closed; no ventilation

note: The descriptions above are based on material presented by Ingalls and Garbe, 1982, and Harvey et al, 1984.

(2) OFFSETS

Automobiles are designed to limit their emissions of specific pollutants to certification standard levels. However, even with proper maintenance, emissions tend, with time, to exceed those standards. With improper maintenance, the emission levels that exceed the standards increase even more. The ratio of the actual emission level to the certified level is referred to as the offset.

For the analyses summarized in Tables 3 through 5, the offsets reflect both properly maintained and malfunctioning (i.e., improperly maintained) vehicles. For the traffic and parking garage scenarios, the offsets shown in those tables reflect average fleet-wide emission levels, taking into account the expected fraction of automobiles that are in a particular malfunction mode (see calculation below); for the personal garage scenario, the offsets for solitary vehicles are used.

Gold (1985) defines a malfunctioning and a properly functioning vehicle as follows: "Malfunction emission rates can be determined by multiplying the maximum likely emission rate under carbon-based standards by the ratio of the average of the prototype vehicle emission rate (without respect to any particular standard) to the average emission rate for properly functioning vehicles. For exhaust emissions, a malfunctioning vehicle is defined as a non-catalyst configuration and a functioning vehicle as one which meets current CO and NO_x standards. For hot-soak emissions, the ratio (i.e., offset) is based on testing...with its (the vehicle's) canister functioning and also with its canister disabled."

Gold (1985) cites other EPA references indicating that "approximately 25 percent of all LDGVs (light duty gasoline vehicles) had experienced in-use catalyst removal, air injection system tampering, or misfueling, resulting in significantly increased exhaust emissions. MOBILE3 projects that operator induced evaporative system disablement averages 2 percent. Evaporative system related failures (involuntary) are estimated at 8 percent. Thus a total of 10 percent of all evaporative systems may be considered to be operating in a malfunction mode."

The formula used to calculate the fleetwide average offsets used in Tables 3 through 5 for traffic and parking garage scenarios is adapted from Gold (1985) as follows:

$$\text{Fleetwide Offset} = (\text{Prop Frac}) \times (\text{Offset}_{prop}) + (\text{Malf Frac}) \times (\text{Offset}_{malf}),$$

where

- Prop Frac = the fraction of the fleet operating properly (0.75 for exhaust, 0.90 for evaporative)
- Offset_{prop} = the offset for vehicles operating properly. MOBILE3 projects a value of 1.37 for exhaust emissions and 1.33 for evaporative emissions
- Malf Frac = the fraction of the fleet malfunctioning (0.25 for exhaust, 0.10 for evaporative)
- Offset_{malf} = the ratio of emission level from an individual malfunctioning vehicle to the level for a vehicle functioning properly. The Offset_{malf} for methanol is dependent on operating mode as follows:

Mode	Ratio
FTP	4.6
HFET	159
Hot-soak	4.8
Idle	34

(3) SOURCES OF DATA FOR TABLES 3 THROUGH 5

Street Canyon, Roadway Tunnel, Expressway (except off-road), and Parking Garage

— Data for *severe* conditions are those given in Gold (1985)

— Data for *typical* conditions are based on the severe level in Gold (1985) multiplied by the ratio of severe/typical emissions for each exposure scenario in Harvey et al (1984). For example, Harvey et al (1984) project that, for a street canyon (and all vehicles performing to certification — i.e., offset of 1.0), typical conditions require 107 g/mile, average light-duty vehicle emission of methanol vapor, to achieve a methanol concentration of 4.5 mg/m^3 while severe conditions require only 16 g/mile to achieve the same ambient concentration (see Harvey et al, Table 4). Thus, given that severe street canyon conditions, with fleetwide average offsets of 1.0, produce 0.25 mg/m^3 (Table 3, street canyon), then typical conditions are expected to produce (16/107) x 0.25 mg/m^3 or 0.04 mg/m^3, as shown in Table 3.

Off-Road Expressway

The off-road values in Table 3 are obtained by applying the given likely certification level (0.023 g/mile) to data in Harvey et al (1984), Table 4. In the latter reference, the authors project that emission levels of 35.7 g/mile, with an offset of 1.0, will achieve an off-road methanol level of 4.5 mg/m^3. To calculate the off-road level for Table 3, one needs to further consider that Harvey et al equate light-duty hydrocarbon emissions to 0.82 of the fleet average, while Gold (1985) assigns a value of 0.62. Since all other values in Tables 3 through 5 are based on Gold (1985), one needs to factor in a correction of 0.82/0.62 to calculate off-road expressway levels that are consistent with the other data shown in the tables. Thus, for an offset of 1.0, the Expressway Off-road Level =

$$(0.023/35.7) \times 4.5 \times (0.82/0.62) = 0.004 \text{ mg/m}^3$$

Personal Garage

All data from Gold (1985)

APPENDIX II

HUMAN STUDIES: REPEATED OR PROLONGED EXPOSURES

Reports of effects from chronic or repeated methanol exposures have appeared infrequently in comparison to reports of acute toxicity. Although details of exposure (duration, concentration) are usually missing, the effects of prolonged exposure are qualitatively very similar to those reported for acute cases, consisting of central nervous and visual disorders. The studies described are divided into case reports and epidemiologic studies.

CASE STUDIES

The first of these was a 1901 report (De Schweinitz, 1901) of a man who became blind after periodic exposure to varnish dissolved in methanol, and the use of methanol to clean his face and arms over a period of three years (also reported in Wood and Buller, case A-25). The Wood and Buller series of case reports in 1904 included several cases that indicated methanol toxicity from extended exposure (Wood and Buller, 1904; Buller and Wood, 1904): failed vision, headache, and vomiting in a man who dyed and cleaned clothes in alcoholic preparations; length of exposure unspecified (Case B-66); deteriorating vision in a man who frequently drank Jamaica ginger (pure methanol) as an alcoholic substitute; an acute dose took his life (Case B-86); a woman who daily took three or four tablespoonsful of Jamaica ginger went practically blind (Case B-89); a woman who, for weeks, used wood alcohol to heat her rheumatic bath and as a cleansing application to face and head presented with impaired vision and partial pallor of the optic nerve (Case C-2); visual loss occurred in a woman who, for two or three months, had used an alcohol-fueled lamp to heat water in a poorly ventilated space; she recovered after her doctor told her to cease exposure (Case C-4); a man who, varnished beer vats for a living, used shellac cut with methanol, and experienced "constitutional symptoms" and "foggy vision" from inhaling the vapors; length of exposure unspecified (Case C-6); and a workman in a cabinet department who cleaned his hands in Columbian spirits to remove shellac suffered from impaired vision (Case C-9).

Severe visual effects were subsequently reported in men exposed to methanol vapor when methanol was used as a paint remover and for mixing shellac (Hawes, 1905); or for varnishing beer vats for periods of 3 to 5 days (Tyson, 1912; Wood, 1912). In each of these instances CNS symptoms (headache, dizziness, nausea, numbness) preceded or accompanied the development of visual symptoms. In a 1905 report (Jelliffe, 1905), CNS symptoms were described in two men who inhaled fumes from shellac dissolved in methanol, but no visual sequelae followed. In none of these reports was the actual level of methanol exposure determined or estimated.

Ziegler (1921) described a man who visited a china cement factory for one hour each day. Methanol was found to be a constituent of the cement. The man had experienced, for several months, failing vision and contraction of visual fields. When visits to the cement factory were stopped, the man slowly recovered and then maintained normal vision. Ziegler (1921) also described a painter who inhaled fumes while varnishing an engine room in a submarine for three days. He was "dizzy" the first day, "hilarious" the second, and "nervous" the third. He also suffered gastric pain and insomnia, soon followed by ptosis (drooping of the eyelid), and blindness. Ziegler also stated that these symptoms were associated with acidosis, although no clinical data were provided. As in the earlier cases, the actual level of exposure was not known.

Humperdink, in 1941, reported the occurrence of mild methanol intoxication with temporary blindness in one laborer employed in a nitrocellulose plant. This worker could presumably have experienced repeated exposures of 1,600 to 10,900 mg/m^3 methanol, which was the amount measured in the air above the weighing station where the worker was employed. The authors noted, however, that over a ten-year period, no other workers had reported any symptoms of methanol toxicity. Burk (1957) also described a case of occupational poisoning attributable to methanol vapor inhalation. This worker had been employed in the methyl alcohol department of a chemical pharmaceutical factory for four years, and had previously complained of visual disorders and asthenia (weakness) of the hands and arms. Upon a two-day exposure to methanol fumes while cleaning a boiler in which crude nicotinic acid was boiled with methanol, he experienced vertigo, nausea, and visual disorders. No information on the airborne concentration of methanol in the air inhaled was provided. Ophthalmoscopic examination showed edema of the optic disc of both eyes. After five weeks, full visual acuity returned.

EPIDEMIOLOGY

A small number of epidemiology studies have been published on methanol exposure, but are generally documented inadequately. The earliest study involves 25 to 30 women who polished wooden lead pencils with varnish made from methanol; many washed their hands in the alcohol to remove the shellac (Tyson, 1912). All of the women reported headaches, and some had gastric disorders during working hours; two reported visual disturbances. They frequently took breaks to get fresh air. The airborne concentration of methanol was unknown. Greenburg et al (1938) studied 19 workers employed in the manufacture of "fused collars." These workers used solutions of three parts acetone to one part methanol to impregnate collars, which were then steam pressed. Concentrations of acetone and methanol in the work room were

measured to be 96 to 108 mg/m^3 and 29 to 33 mg/m^3, respectively, and a "strong odor" of solvent was perceptible. The shortest period that any of these workers had spent fusing collars was nine months, and the longest was two years. No central nervous system or visual anomalies in any of these workers were reported.

In 1955, Kingsley and Hirsch reported frequent and persistent headaches in clerical workers located close to spirit duplicating equipment that used methanol-based duplicating fluid. The most severe headaches were reported to occur in personnel who actually operated the equipment. The onset of symptoms coincided with the beginning of cooler weather, which required the closing of windows and doors, thus inhibiting ventilation. No visual effects or other permanent sequelae were reported. Kingsley and Hirsch (1955) measured methanol concentrations as high as 490 mg/m^3 in the air surrounding the duplicating equipment after 60 minutes of operation, and approximately 130 mg/m^3 about ten feet away from the device. The methanol concentration around the device in question always exceeded 260 mg/m^3. No information on the number of employees exposed or affected, or on the actual duration of methanol exposure, was provided in this report.

More recently, the National Institute for Occupational Safety and Health (NIOSH, 1981) reported that 45 percent of "spirit" duplicating machine operators at the University of Washington experienced "some" symptoms (blurred vision, headache, nausea, dizziness, and eye irritation) consistent with the toxic effects of methanol. Apparently, no information on the actual level of duration of methanol exposure among these employees was collected. When NIOSH measured airborne methanol concentration for 25 minutes in the vicinity of the duplicators when windows and doors were open, the average was 1,330 mg/m^3.

In 1984, Frederick et al of NIOSH published a study of teacher aides who worked at or near spirit duplicators that used a 99% methanol duplicator fluid. The exposures ranged from 1 hour per day for 1 day per week to 8 hours per day for 5 days per week, and had been occurring presumably for about three years. Ventilation was either inadequate or totally lacking. Since the introduction of the equipment, the aides began to experience headaches, dizziness, and eye irritation while operating the machines or working near them. Fifteen-minute breathing zone samples near 21 operating machines contained between 475 and 4,000 mg/m^3 (1,380 $\pm$ 745(SD) mg/m^3) of methanol vapor; 15 of these samples exceeded NIOSH's recommended 15-minute standard of 1,050 mg/m^3 (or 800 ppm). The existing ventilation, when operating, lowered the levels around selected machines by an average of 58% (range: 7 to 89%); even so, methanol concentrations exceeded the 260 mg/m^3 8-hr standard for at least half of the duplicators tested. When NIOSH-fabricated enclosures were added, the unventilated values dropped buy an average 96% (range: 90 to 99%). The aides also were exposed while collating and stapling papers impregnated with the fluid up to three hours earlier, and these exposures ranged from 235 to 1,140 mg/m^3.

A health questionnaire survey was conducted among 84 teacher aides and 302 teachers, who served as a comparison group. Teachers, though working in the same school, probably spend significantly less time near the duplicators (and less time collating) than the aides. All aides and teachers surveyed were female. Sixty-six (79%) of the aides responded (mean age 39.8); their responses were compared to those of 66 randomly selected teachers (mean age 37.5). The respondents provided data on the prevalence of 22 specific symptoms that they experienced in the month preceding the survey. The list included symptoms considered both related and unrelated to methanol's effects. Four of the 22 symptoms were significantly higher in the aides, and all have been associated with methanol toxicity: headache, dizzy/lightheaded, blurred vision, and nausea/upset stomach (Table I-1). No other symptoms registered significant differences between the two groups, although positive trends appeared evident for burning/itching/tearing of the eyes (17 of 66 aides versus 8 of 66 teachers) and skin problems (7 versus 1, respectively).

Criteria were established to define a positive case of methanol toxicity (Table I-2). With these criteria, 30 aides and 16 teachers qualified, a difference that is significant ($p < .025$). Finally, the investigators determined that the case attack rate increased, for both aides and teachers, as a function of percent of time spent at the duplicator each week.

This study clearly stands apart from all others available because it provides data on ambient concentrations, duration of exposure, health status, and the relation between case attack rate and work-time exposed. The results suggest that chronic effects may occur when methanol concentrations exceed the TLV of 260 mg/m^3. The effects in this study are similar in nature but appear less severe than those from acute intoxication.

Though of value, this study, nevertheless, has several features that prevent one from drawing a definitive causal association between methanol exposures and the effects reported. First, the study was conducted in response to the teacher aides' complaints, and thus the questionnaire data may have contained responder bias. Second, the data were based on symptom reporting only, whereas additional clinical investigations may have helped to further define the effects. Finally, information is not presented to exclude the possibility that the symptoms might have arisen from other chemicals or solvents that may have been in the teacher aides' environment. Despite these shortcomings, the study by Frederick et al is of relevance.

Other studies have measured methanol and formate in the blood and urine of workers exposed during the 8-hour day to between 100 and 200 mg/m^3 of methanol vapors (Baumann and Angerer, 1979; Heinrich and Angerer, 1982). Although these studies were predicated on issues of occupational health related to methanol exposure, no health data are provided. In none of these studies do the investigators imply that the workers studied had suffered health effects.

Table II-1 Symptoms Significantly ($p < .05$) More Prevalent in Teacher Aides than Teachers

Symptom	# Teachers (n = 66)	# Aides (n = 66)	Ratio
Headache	12	23	2
Dizzy/lightheaded	1	20	20
Blurred vision	1	15	15
Nausea/upset stomach	4	12	3

Adapted from: Frederick et al. 1984

Table II-2 Criteria for Defining Methanol Toxicity

1. Visual changes or blurred vision
2. One acute symptom (headache, dizziness, numbness, giddiness, nausea, or vomiting) and one chronic symptom (usually tired, muscle weakness, trouble sleeping, irritability or poor memory)
3. Two acute symptoms
4. Three chronic symptoms

Adapted from: Frederick et al. 1984

APPENDIX III

ACGIH TLV FOR METHANOL

Reprinted, by permission, from: American Conference of Governmental Industrial Hygienists, Inc. (ACGIH). Documentation of the Threshold Limit Values and Biological Exposure Indices. Fifth edition. Cincinnati, OH. 1985.

METHYL ALCOHOL

CAS: 67-56-1

Methanol

CH_3H

Skin

TLV-TWA, 200 ppm (≈ 260 mg/m³)
TLV-STEL, 250 ppm (≈ 310 mg/m³)

Methyl alcohol is a mobile, highly polar, flammable liquid. Its physicochemical properties include:

Molecular weight: 32.04
Specific gravity: 0.7915 at 20°C
Melting point: −97.8°C
Boiling point: 64.5°C
Vapor pressure: 92 torr at 20°C
Vapor density: 1.11 (air = 1)
Closed cup flash point: 54°F (12°C)
Autoignition temperature: 878°F (470°C)
Explosive limits: 6.7% and 36.5% by volume in air

It is miscible with water, ethyl alcohol, ether, and many other organic solvents.

Methanol is used as a solvent for nitrocellulose, ethyl cellulose, and various natural and synthetic resins; as a denaturant for ethyl alcohol; as an antifreeze; and in the manufacture of formaldehyde and many other chemicals, notably methyl derivatives.

According to Henderson and Haggard,[1] methanol is slowly eliminated from the body, hence repeated exposures result in an increasing concentration in blood and tissue. McNally[2] stated that occupational methanol poisoning has frequently caused death or blindness. Several cases resulted from work in confined spaces, e.g., varnishing beer vats. Headaches and blurred vision were reportedly frequent symptoms. He believed that 8 grams would seriously affect the eyes. (Such a dose could result from inhalation of 800 to 1000 ppm for eight hours.) According to McNally, workroom concentration of 500 to 6000 were found; he recommended that levels be kept below 1 ppm.

Browning[3] stated that cases of chronic poisoning from repeated exposure to methyl alcohol vapor were manifested by conjunctivitis, headache, giddiness, insomnia, gastric disturbances and failure of vision. In one fatal case of occupational methanol intoxication by inhalation, a female worker was exposed about 12 hours.[4] A post-event study of the process revealed methanol vapor concentrations ranging from 4000 to 13,000 ppm. Henson,[5] in a review of methanol toxicity, mentioned a report of chronic poisoning, with marked diminution of vision, resulting apparently from exposure at 1200 to 8000 ppm for four years. Other workers in the area were not affected. Henson also recorded reports of headaches among workers exposed at 300 ppm during the operation of duplicating machines.

Most of the serious cases of methanol poisoning which have been reported during the last 40 years, many of them fatal, others involving permanent or temporary loss of vision, resulted from the ingestion of methyl alcohol in the belief that it was ethyl alcohol.

A study of the wood heel industry in Massachusetts[6] showed average methanol vapor concentrations ranging from 160 to 780 ppm, with no definite evidence of injury to the exposed workers. McAllister[7] reported concentrations between 400 and 1000 ppm in spirit duplicating processes. No mention was made of symptoms or complaints, but these concentrations were considered excessive.

Savers and co-workers[8] observed no symptoms in dogs exposed daily (seven days a week) for 379 days at concentrations between 450 and 500 ppm. Leaf and Zatman,[9] after studying the rates of absorption and excretion of methanol, concluded that at 3000 ppm accumulation in the body would occur, and that the maximum safe concentration for occupational exposure was 300 ppm.

Cook,[10] on the basis of the Savers study,[8] recommended a limit of 200 ppm. It is probable that this value incorporates a fairly large margin of safety against serious toxic effects.

According to the NIOSH criteria document for methyl alcohol,[11] the signs and symptoms most characteristic of methanol poisoning are various visual disturbances and metabolic acidosis.[11] The NIOSH review of the literature failed to reveal any epidemiologic surveys sufficiently comprehensive to bear significantly on the workplace environmental limit. A report by Kingsley and Hirsch[12] indicated severe recurrent headaches in workers exposed to methyl alcohol in concentrations between 200 and 375 ppm. Diminution of vision was reported from airborne methyl alcohol concentrations of 1200 to 8300 ppm.[11]

NIOSH therefore recommended a TWA standard of 200 ppm for methyl alcohol, the same as the time-weighted average TLV of long standing. A fifteen minute ceiling of 800 ppm, well above the TLV-STEL of 250 ppm, was recommended.[11]

Other recommendations: ANSI (1944) 200 ppm; Sweden (1975) and West Germany (1974) 200 ppm; Czechoslovakia (1969) and East Germany (1973) 75 ppm; USSR (1972) 4 ppm.

References

1. **Henderson, Y. and H.W. Haggard:** *Noxious Gases*, p. 218. Reinhold Publishing Corp., New York (1943).
2. **McNally, W.D.:** *Toxicology*, p. 615. Industrial Medicine, Chicago, IL (1937).
3. **Browning, E.:** *Toxicity and Metabolism of Organic Solvents*, pp. 311-323. Elsevier Publishing Co., Amsterdam (1965).
4. Unpublished report in files of Massachusetts Division of Occupational Hygiene (1932).
5. **Henson, E.V.:** *J. Occup. Med. 2*:497 (1960).
6. **Massachusetts Division of Occupational Hygiene:** Health Hazards of Wood Heel Covering (1937).
7. **McAllister, R.G.:** *Am. Ind. Hyg. Assoc. Q. 15*:26 (1954).
8. **Sayers, R.R., W.P. Yant, H.H. Schrenk et al:** *U.S. Bureau of Mines Report of Investigations No. 3617* (1942).
9. **Leaf, G. and L.J. Zatman:** *Brit. J. Ind. Med. 9*:19 (1952).
10. **Cook, W.A.:** *Ind. Med. 14*:936 (1945).
11. **NIOSH:** *Criteria for a Recommended Standard — Occupational Exposure to Methyl Alcohol.* DHEW Pub. No. (NIOSH) 76-148 (1976).
12. **Kingsley, W.H. and F.C. Hirsch:** *Compen. Med. 40*:7 (1954-55).
13. **Humperdinck, K.:** *Arch. Gewerbepathol. u. Gewerbehyg. 10*:569 (1941).

APPENDIX IV

DISCUSSION OF RUSSIAN HUMAN CLINICAL STUDIES

Russian investigators published papers in 1959 and 1967 that claimed neurobehavioral effects in humans who were exposed to very low exposure levels of methanol vapors (less than 12 mg/m^3). In the first paper, Chao (1959) measured the threshold of olfactory perception and dark adaptation (or light sensitivity). The second, by Ubaydullayev (1967), included both of these measures in addition to the EEG conditioned reflex threshold. These studies are presented and analyzed in detail in the discussion that follows.

OLFACTORY THRESHOLD

Of the three parameters tested in the two studies, olfactory threshold is the only one that other investigators also have tested. These other studies, two in all, report olfactory thresholds between two and three orders of magnitude higher than Chao and Ubaydullayev. Scherberger et al (1958) studied thresholds in three subjects and reported a minimum identifiable concentration of 1,950 mg/m^3. May (1966) studied 16 subjects and reported a minimally perceptible level of 7,670 mg/m^3.

Several problems, related to the procedural aspects of olfactory testing, call into question the results of the two latter studies. Basically, the sensory threshold is the level of exposure that is detected accurately 50 percent of the time. Threshold determination depends on the systematic acquisition of subject-specific curves that plot response as a function of exposure concentration.

Another important consideration in odor-threshold detection is the nature of olfactory physiology. The presentation of olfactory stimuli must be carefully controlled in order to assess accurately whether or not the threshold actually has been reached. With closely repeated or continuous exposures to an olfactory stimulus, the ability to detect it rapidly decreases, a phenomenon known as olfactory fatigue. When olfactory fatigue is present, progressively higher concentrations must be presented in order for the subject to detect the chemical. Naturally, this would prevent the accurate detection of a minimum threshold. Neither the Scherberger nor the May studies employed adequate paradigms for threshold detection or protected against olfactory fatigue. In the Scherberger study, no experimental details were provided, including whether several exposure levels were tested for each subject or whether subjects were exposed to high concentrations immediately prior to lower ones; also, subjects may have experienced olfactory fatigue. Likewise, the threshold in the May study should be viewed as unreliable because there is a strong likelihood that olfactory fatigue affected the results.

In contrast to these reports, both the Chao (1959) and Ubaydullayev (1967) studies apparently used more appropriate methodology for the detection of odor thresholds. According to a comprehensive review of behavioral toxicology paradigms used in the USSR (NIOSH 1976b), Russian investigators use paradigms prescribed by a committee affiliated with the USSR Academy of Sciences. Therefore, although methodology in Russian research papers, such as the ones cited above, is often poorly explained, the procedures used are standardized, and reference can be made to the author of the method or to key papers. Standard protocols for a variety of tests, including olfactory thresholds, have been described by NIOSH (NIOSH 1976b). Those descriptions have been relied upon here to interpret the English translations of the Chao and Ubaydullayev studies.

In both Russian olfactory studies, two sniffing cylinders, one that contained pure air, and the other that contained either a methanol-air mixture or pure air, were given to subjects who were asked to sniff freely from each until ready to indicate which contained the methanol mixture. Each concentration was presented at least three times. The minimum perceptible concentration for each subject was taken as the lowest concentration detected in at least two out of three trials. Presumably, the criterion for the maximum imperceptible concentration also was correct detection on at least two out of three trials, although that is not clearly delineated in the standard protocols. Both a minimum perceptible and a maximum imperceptible methanol concentration were reported.

Using this paradigm, in the Chao (1959) study the minimum detectable airborne concentration of methanol was found to range from 4.3 to 11.1 mg/m^3 and the maximum imperceptible airborne concentration was found to range from 3.7 to 10.5 mg/m^3. In the Ubaydullayev (1967) study, the maximum imperceptible methanol concentration ranged from 3.9 to 9.7 mg/m^3 and the minimum perceptible concentration ranged from 4.5 to 10.3 mg/m^3.

Although the Russian studies apparently adhered to an appropriate methodology, the results should be viewed as provisional. An important substantive concern that forces this conclusion is the investigators' failure to document the purity grade of the methanol used. Impurities can have a marked effect on olfaction, producing false-positive results at methanol levels that are actually sub-threshold. Despite the shortcomings of the Scherberger and May studies, the large discrepancy of the results from these and the Russian set is difficult to reconcile. The possibility that impurities may have skewed the Russian results cannot be ruled out. This opinion also was expressed in the 1976 NIOSH document on occupational exposure to methanol (NIOSH, 1976a). One further issue that applies to the olfactory experiments, as well as to the dark adaptation and EEG tests, is the failure of either Chao or Ubaydullayev to describe the manner in which methanol vapor concentrations were measured. Instrumentation,

measurement technique, and calibration procedures each can serve as source of error, and distort test results. Regardless of the value of the "true" threshold, olfaction is an indication of sensory stimulation, not a toxic response.

DARK ADAPTATION

In simple terms, a dark adaptation curve describes the threshold level of light one perceives as a function of time in the dark. Normally, the threshold decreases with time (i.e., sensitivity to light increases) as adaptation continues and ultimately levels to a final value (Guyton, 1981). Cones (receptors specialized to discriminate color) and rods (do not discriminate color, specialized for dark vision) typically adapt differently; rods adapting slow over the course of 30 to 60 minutes, and cones adapt within 10 minutes, but to a much lower sensitivity than the rods. Thus, depending on test conditions, adaptation curves may demonstrate a "rod-cone break." For example, if the test spot of light is directed to the retinal periphery, which is relatively cone-poor, then the break will not occur; similarly, if the pre-adapting stimulus (the lighting condition prior to dark adaptation) is itself dim, cone adaptation already may have concluded prior to the adaptation test, and again no break will appear. The eye's sensitivity to light can change by a factor of between a half-million and a million, that is, between 5.7 and 6.0 $\log_{10}$ units. Of these, photochemical receptivity of rods and cones account for about 4.4 (x 25,000) and pupillary adjustments for about 1.5 (x 30); an additional fraction of a log unit is associated with intermediate neuronal levels in the retina. Tests of dark adaptation typically probe for photoreceptivity in the retina, and uncontrolled changes in pupillary size. Extraneous factors that are distracting to the subject can confound a test's results.

Dark adaptation is inherently difficult to measure for two reasons. First, it is continually changing, so the measurement must be of short duration. Second, one uses light presentation to measure dark adaptation, and if the test light is too bright it will alter the course of adaptation. To mitigate these problems, the Russian investigators chose a von Bekesy tracking technique. For each measurement, the intensity of the test flash is increased until the subject signals that it is seen. The light stimulus then decreases in intensity until the subject signals that it is no longer visible. The light threshold is taken as the mean of these two inflections.

In both Russian studies, the subjects were exposed to methanol from the 15th to the 20th minute of dark adaptation. Dark adaptation curves are usually dynamic during this time frame and, therefore, are maximally sensitive to acute effects. Chronic conditions, such as vitamin A deficiency, manifest over the entire time course of dark adaptation, or in the final level of sensitivity.

As in the olfaction experiments, the dark adaptation studies probably were performed with standardized procedures (NIOSH, 1976). Ubaydullayev reports using an ADM adaptometer, which is one of two adaptometer recommended for use in the Soviet Union (Chao's equipment was unspecified). In the procedure, the stimulus, transilluminated with an incandescent lamp, locates binocularly 12 degrees to the right of fixation (i.e. 12 degrees from the fovea, the point of sharpest vision in the retina). This type of stimulation usually produces a curve with some evidence of rod and cone components, with the transition occurring late in the first ten minutes of adaptation (as mentioned before, the rod-cone break will not appear with a sufficiently dim pre-adapting stimulus).

The Chao study and Ubaydullayev study each tested dark adaptation in three subjects. The subjects in the Ubaydullayev study were between 18 and 25 years old; no characteristics of Chao's subjects were given. In Chao's experiments, adaptation for each subject was measured at five different methanol concentrations (0, 1.8 to 2.4, 3.3 to 3.7, 4.3 to 4.7, and 5.7 to 6.5 mg/m^3); according to the translation, "59 determinations were done in all" and "each concentration was examined several times." In Ubaydullayev's study, four concentrations were used (0, 3.06, 3.53, and 4.11 mg/m^3), and "tests were made daily on each individual under identical conditions and at a standardized time of day." Further, "the physiological background — i.e. the normal curve of eye adaptation to the dark or sensitivity to light — was determined on 8 successive days by 15- and 20-minute pure air inhalations." These are the only details provided.

Important information that neither investigator provided concerns the number of curves run for each individual for each concentration (or how they varied) and the time points along each curve at which light detection was measured; nor did they discuss the pre-adapting stimulus, the size of the stimulus on the retina, or how they controlled fixation, all of which may influence the rod-cone break.

Both investigators reported seemingly consistent results. Chao claimed an effect at 3.3 mg/m^3, but none at 2.4 mg/m^3, and Ubaydullayev claimed an effect at 3.53 mg/m^3, and none at 3.06 mg/m^3. Actual data, however, are shown for only a single subject in each study. Furthermore, the time-course of adaptation for the curves shown in both studies is quite unusual for this kind of test, displaying an upwardly concave shape in the first few minutes. Generally, dark adaptation proceeds rapidly in the first few minutes and the shape has a downward concavity.

The few data shown from the two studies are inconsistent. In Ubaydullayev's study, 3.53 mg/m^3 produced an immediate rise in sensitivity (methanol is presented at the 15th minute of adaptation) that peaked at 20 minutes of adaptation (when methanol exposure ceased) and returned to control values at 25 minutes; 4.11 mg/m^3 produced an immediate depression that lasted until 30 minutes. The test terminated at 40 minutes. Chao's results showed an opposite trend; 3.3 to 3.7 mg/m^3 produced a subtle drop in sensitivity from the time that methanol was introduced to the end of the test (60 minutes), whereas 4.3 to 4.7 mg/m^3 produced a small initial rise in sensitivity followed by a dip below control about 20 minutes later, which persisted to the end. At 5.7 to 6.5 mg/m^3, Chao's data

accentuated the effects reported at 4.3 to 4.7 mg/m^3. If methanol produced a distinct acute effect at these concentrations, one would expect a consistent set of data from "replicate" experiments. The results presented offer no such possibility.

Several other factors are germane to this discussion. First, though olfactory sensations do not directly influence the photoreceptivity of rods and cones, they may distract a subject to the extent that his or her perception of light is mildly altered. Since the thresholds for olfaction of the methanol (and/or its impurities) overlap the levels that putatively affect dark adaptation, the possibility of distraction cannot be dismissed. Second, the reader should bear in mind that if methanol is affecting visual sensitivity in these experiments, the effect bears no correspondence to the classic visual toxicity seen after a one day latency. Post-latency toxicity results from a methanol metabolite, probably formate, whereas, in the kinds of experiments described here, the alcohol itself would be responsible. Finally, the amount of methanol absorbed into the body during the five-minute exposure is insignificant compared to background levels in the body. To illustrate, a "typical" 70 kg man with 45 liters of body water (methanol distributes uniformly to body water) breathing and totally absorbing 10 mg/m^3 methanol for five minutes, would raise his concentration of methanol by 0.008 mg methanol/liter body water. In contrast, background blood levels of methanol are around 0.75 mg/l or more than 100 times greater than the amount contributed during the Russian dark adaptation, which were all run below 12 mg/m^3. How this minor amount may directly affect central nervous function must remain open to conjecture.

To conclude, altered dark adaptation may constitute an "adverse effect." The apparent similarity of results from the two Russian studies published eight years apart by different investigators is noteworthy. However, if the protocols, reagents, and instrumentation remained standardized in the period that covered both studies, then one could expect similar, but not necessarily valid, results.

EEG-CONDITIONED REFLEX THRESHOLD DETERMINATION

Ubaydullayev (1967) also examined conditioned alpha rhythm amplitude using an electroencephalogram (EEG) in six subjects exposed to methanol. The methods were poorly described in the paper and, consequently, the following comments rely, again, on the NIOSH (1976b) review of Russian neurobehavioral toxicology methods.

According to NIOSH (1976b), a depression in cortical alpha wave activity is used as a conditioned response in these experiments. Various concentrations of a volatile chemical (methanol) are presented as conditioned stimuli, and the onset of a light is presented as the unconditioned stimulus. In an experimental conditioning paradigm such as this, the unconditioned stimulus (light) is one that normally elicits the conditioned response (depressed alpha wave activity). When both the unconditioned and conditioned stimulus (light and methanol, respectively) are presented together often enough, the conditioned stimulus alone (methanol) develops the ability to elicit the response. Thus, the fact that a stimulus, such as a certain methanol concentration, can be conditioned, is evidence that it has been detected. The lowest concentration of a chemical that can be conditioned is reported as the EEG-conditioned reflex threshold.

In the Ubaydullayev (1967) study, conditioning was attempted in the six subjects most sensitive to olfactory stimulation. They were exposed to methanol concentrations of 1.01, 1.17, and 1.46 mg/m^3. The lowest concentration of methanol that successfully conditioned was 1.17 mg/m^3, eliciting the response in two subjects. All six subjects exhibited depressed alpha wave amplitude, displaying the conditioned response when presented with 1.46 mg/m^3 methanol.

Detection, as reflected by altered alpha rhythms, occurred below threshold levels for olfaction, a finding that is not uncommon. Such a response is indicative of an organism's ability to detect the presence of a substance, and provides evidence of a normally functioning nervous system.

Several of the concerns voiced earlier linger. Details about the purity grade of methanol, the subjects, the exposure protocol, and test data are lacking. At the levels used, the accumulation of methanol in the subjects is, in all likelihood, negligible compared to the normal methanol body burden. Finally, nowhere in any of these studies do the investigators guarantee that both they and the subjects remained blind to the exposure condition.

APPENDIX V

STUDIES OF REPEATED OR PROLONGED METHANOL EXPOSURE IN NON-PRIMATES

RODENTS:

Because of the superiority of non-human primates as experimental models of human methanol toxicity, few experiments on the biologic effects of methanol have been conducted using rodents in the past 30 years. In a study by Skirko et al (1976) (a Russian study cited in Rowe and McCollister, 1982), rats received oral doses of 10, 100, or 500 mg/kg/day for one month and were reported to show liver changes characterized by focal proteinic degeneration of hepatocytic cytoplasm, changes in the activity of some microsomal enzymes, and enlarged hepatic cells. [In another Russian study, rabbits exposed to 61 mg m^{-3} methanol for six months (duration of exposure per day not given) were reported to have ultrastructural changes in the photoreceptor cells and Muller fibers (Vendilo et al, 1971, a study cited in Rowe and McCollister, 1982).] The reliability of either of these reports remains to be established through critical review of translated articles. In an English article, White et al (1983) reported that exposure of rats (Sprague-Dawley) to airborne methanol concentrations of 260, 2,600, or 13,000 mg/m^3 for as long as six weeks caused no signs of lung inflammation or irritation. Histologic analyses of lung tissue were not conducted.

In a subchronic inhalation study, Sprague-Dawley rats were exposed for 4 weeks (6 hours per day, 5 days per week) to 650, 2,600 and 6,500 mg/m^3 of methanol vapor (Andrews et al, 1987). The animals were observed twice daily for signs of toxicity, and were given detailed physical examinations each week, and ophthalmoscopic examinations at pre-test and at termination. After sacrifice, the animals' organs were examined and weighed, and selected tissues from all animals in the control and high-exposure groups were examined microscopically. These included nasal turbinates, trachea, lungs, trachea, esophagus, liver, and the eye and optic nerve. The investigators report no effects, except for increased discharges about the eyes and nose. The only dose-related effect observed was mucoid nasal discharge, which the investigators believe is reflective of upper respiratory tract irritation. Though stating that this effect was dose-related, Andrews et al provide no dose-effect data on this finding. No other treatment-related effects were observed in this study.

Behavioral toxicity associated with exposure to low concentrations of methanol in laboratory rats was reported in two studies in the Russian literature (Chao, 1959; Ubaydullayev, 1967). Chao (1959) exposed groups of ten rats (of unspecified sex and strain) to methanol vapor at concentrations of 0, 1.77, and 49.77 mg/m^3 for 12 hours per day ("excluding days off") for 3 months. The relationship of flexor to extensor chronaxy was measured at unspecified intervals. According to a review of behavioral toxicology paradigms used in the USSR (NIOSH 1976b), "chronaxy is the minimum time necessary for a stimulus of twice the absolute threshold intensity to evoke a response" and is measured as muscle contractions in response to an electric current applied to the animal's hind leg. Normally, the flexor chronaxy is shorter than the extensor chronaxy, and their ratio is stated to be a relatively stable one. According to the NIOSH review, certain toxic agents have been found to reverse this relationship (NIOSH 1976b). Although the chronaximetry method as used by Chao (1959) and Ubaydullayev (1967, see below) is poorly described in both published studies, the methodological details provided are consistent with the standardized methodology summarized by NIOSH (1976b).

Chao (1959) reported that the average chronaxy ratio for rats in the high dose group significantly differed from that in the controls at week eight of exposure. The average chronaxy ratio was stated to have returned to normal during the recovery period. Effects in the low-dose group were reported to be insignificant. Although the investigators reported that dose groups consisted of 10 animals per group, they did not indicate the number of animals tested per testing interval, or the frequency of measurement of chronaxy ratios. Data were presented only graphically and actual chronaxy ratios and results of statistical analyses were not provided.

Chao (1959) also reported certain histopathological changes in the high-dose group but not in the low-dose group. The lesions included "poorly defined changes in the mucous membranes of the trachea and bronchi," hyperplasia of the submucosa of the trachea, slight lymphoid infiltration, swelling and hypertrophy of the muscle layer of pulmonary arteries, slight degenerative changes to the liver, and changes in the neurons of the cerebral cortex. A list of tissues examined histopathologically, the number of animals per group subject to pathological examination, and the incidence of tissue lesions were not provided.

Ubaydullayev (1967) exposed groups of 15 male rats (strain not specified) to methanol at average air concentrations of 0, 0.57, and 5.31 mg/m^3 for 24 hours per day for 90 days. Motor chronaxy ratios were measured at 10-day intervals in five rats per group. Ubaydullayev reported that the high-dose group "manifested statistically reliable changes" in the motor chronaxy ratio beginning at week 6, and that the ratio returned to normal by the end of the recovery period (length of recovery period is not specified). Average values for the chronaxy ratios for the three groups over the study were presented graphically, but the actual data and statistical analyses were not provided.

Urinary coproporphyrin levels, whole blood cholinesterase activity, and levels of total protein and protein fractions in blood serum also were measured in 5 rats of each group (Ubay-

dullayev 1967). In the high-dose group, the investigator reported a decrease in urinary coproporphyrin levels and cholinesterase activity. Blood serum albumin levels dropped and levels of beta- and gamma-globulins increased in the high-dose group compared to the controls. These parameters were reported to have returned to normal after cessation of exposure. No effects were observed in the low-dose group. The investigator presented average values for the control, low-dose, and high-dose, but did not provide any statistical analysis of the data.

The results reported by Chao (1959) and Ubaydullayev (1967) do not provide adequate evidence of an association between neurobehavioral effects and low-level exposure to methanol in laboratory animals. Both studies are limited by the use of small numbers of animals per dose group, as well as insufficient reporting of experimental methods, study results, and statistical analyses. Furthermore, the biological significance of changes in the chronaxy ratio is uncertain. Although measurement of the chronaxy ratio in rats appears to be a standard protocol for assessing neurobehavioral toxicity in Russian research, it is not a toxicological measure reported in the U.S. literature.

DOGS:

Sayers et al (1944) exposed two dogs to about 13,000 mg/m^3 methanol for about three minutes at hourly intervals eight times daily for 100 days, a total of 800 brief exposures. Both dogs were reported to have survived the exposure and exhibited no symptoms or unusual behavior or visual toxicity attributable to methanol poisoning. In an earlier study (Sayers et al. 1942), four dogs were exposed to airborne concentrations of methanol from 585 to 650 mg/m^3, eight hours per day, seven days per week for 379 days in a continuously ventilated chamber. The authors performed a wide range of hematologic determinations and ophthalmoscopic examinations. No adverse effects of any kind were reported.

POTENTIAL REPRODUCTIVE, TERATOGENIC, OR CARGINOGENIC EFFECTS OF METHANOL EXPOSURE

Three studies have been reported in which the reproductive or teratogenic effects of methanol in nonprimate species were investigated.

Cameron et al (1984) exposed mature male rats (Sprague-Dawley) to airborne methanol concentrations of 260, 2,600, or 13,000 mg/m^3 for one, two, four, or six weeks and examined them for alterations in circulating free testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Significantly decreased levels of circulating free testosterone were observed among rats exposed to 260 mg/m^3 for two and six weeks and to 2,600 mg/m^3 for six weeks. The high dose group (13,000 mg/m^3) showed no change, however. The authors interpreted this as evidence that methanol exposure had lowered testicular production of testosterone. In addition, significant increases in circulating LH were observed after six weeks of exposure to 13,000 mg/m^3. No changes in FSH levels were observed.

Nelson et al (1985) administered 0, 6,500, 13,000, or 26,000 mg/m^3 methanol to groups of approximately 15 pregnant Sprague-Dawley rats for seven hours per day on days 1 through 19 of gestation (for 26,000 mg/m^3, days 7 through 15 only). The blood levels of methanol in the 26,000 mg/m^3 group ranged from 8.34 to 9.26 mg/ml after one day of exposure and from 4.84 to 6.00 mg/ml after ten days of exposure.

The highest concentration of methanol produced slight maternal toxicity (unsteady gait) and a high incidence of congenital malformations, predominantly extra or rudimentary cervical ribs and urinary or cardiovascular defects. Among 15 litters exposed to 26,000 mg/m^3, 14 contained at least one fetus with a skeletal malformation, and 10 contained at least one fetus with a visceral malformation. These incidences of malformation were significantly different from the control group ($p < .05$), which had no skeletal or visceral malformations in any of 15 litters. Similar malformations were seen in the group exposed to 13,000 mg/m^3, but the incidence were not significantly different from those of controls. No increase in malformations was observed in the group exposed to 6,500 mg/m^3, which the authors interpret as a no-effects level for this test system.

It was noted when reviewing this study that different incidences of visceral malformations were reported in the text than were reported in the accompanying tables. Those inconsistencies should be resolved before accepting the reliability of this paper. Moreover, the occurrence of maternal toxicity in the significantly affected group compromises an interpretation of the teratogenic effects as being solely the result of in utero methanol exposure.

Infurna and Weiss (1986) examined early behavioral development in Long-Evans rats exposed prenatally to methanol. The study focused on suckling and nest-seeking behaviors of the neonates. Treatment consisted of providing pregnant rats with a drinking solution containing 2% (volume ratio) methanol; one group received this treatment during gestational days 15 through 17, and a second group received the treatment during days 17 through 19. This treatment resulted in an average methanol consumption of 2.5 g/kg/day; controls received normal water. Increased latency to suckling behavior was observed in pups from both groups of methanol-treated dams, when the pups were tested 24 hours after their birth. In addition, pups in both groups exposed prenatally to methanol displayed a lower efficiency in seeking and reaching their home area when tested on postnatal day 10. Methanol treatments did not affect litter size, birthweight, weight gain during the preweaning period, infant mortality, or day of eye opening. Also unaffected were duration of gestation, weight gain in the third week of gestation, and maternal behavior on the day of parturition. The authors conclude that methanol "can be defined as a behavioral teratogen in rats, since no other signs of toxicity were apparent either in the mothers or the offspring."

The behavioral effects noted in this study occur at tissue levels of methanol lower than those associated with teratogenesis in the study by Nelson et al (1985), and may be of potential significance. However, maternal exposures to methanol during the three-day treatment periods in the Infurna and Weiss (1986) study (2.5 g/kg/day) are equivalent to at least 2,500 daily human exposures to methanol vapors under expected worst-case conditions. (As discussed in Section IV of this report, the added body burden of methanol resulting from worst-case exposure will be less than 1 mg/kg.) Clearly, dose-effect data on the parameters studied by Infurna and Weiss would help clarify whether or not humans may experience similar effects at or near expected ambient exposure levels. Such studies may be of particular value as they focus on endpoints representative of potentially subtle effects to the central nervous system.

CARCINOGENICITY AND MUTAGENICITY

There have been no studies reported in the peer-reviewed literature on the potential carcinogenicity of methanol in laboratory animals. As mentioned in Section III, the New Energy Development Organization (in Japan) sponsored chronic carcinogenesis bioassays in which mice (18 months) and rats (24 months) were exposed to 13, 130, and 1,300 mg/m^3. The report issued from that study contains insufficient detail to allow for critical review.

Methanol has not been extensively tested for mutagenicity. It produced negative results in Schizosaccharomyces pombe with or without a microsomal activating system from mouse liver (Abbondandolo et al, 1980). Even with the addition of an activating system, this study did not properly address the mutagenicity of methanol metabolites. This is because the microsomal activating system used was from mice whose metabolic profile may be dissimilar to that in humans. In addition, soluble enzymes such as alcohol dehydrogenase, which are required in methanol metabolism in humans, are removed from microsomal preparations.

APPENDIX VI

EFFECTS OF FORMIC ACID (FORMATE)

Formic acid is the second oxidation product of methanol. It is a normal cellular constituent involved in numerous metabolic reactions. It is also a natural constituent of many foods. Formic acid is used in foods as a flavoring adjunct, brewing antiseptic, and preservative (FASEB, 1976). In a health evaluation conducted by FASEB (1976) for the Food and Drug Administration, the committee members concluded that there was no evidence that suggested that exposure to formic acid or sodium formate, when used as a food additive, would pose a hazard to the general public.

Effects Following Acute Formic Acid Exposure: Formic acid is a primary irritant that can cause severe damage to the skin, eye, or respiratory tract (Guest et al, 1982). Workers exposed to 15 ppm (28 mg/m^3) of formic acid in the air have complained of nausea (ACGIH 1985b). Rats (Wistar) exposed to 38 mg/m^3 of formic acid vapor 6 hours per day for 3 or 8 days were conspicuously inactive during the exposure period, but displayed no clinical signs of toxicity at the time of sacrifice (Zitting and Savolainen, 1980). Slight alterations in glutathione levels in the brains, livers, and kidneys of the rats were observed along with minor effects on drug metabolizing enzymes. At physiologic pH, formic acid dissociates to formate and a hydrogen ion. As discussed in the body of this report, high formate levels in the body have been associated with ocular toxicity in monkeys and humans after high methanol exposures.

Effects Following Repeated or Prolonged Formic Acid Exposure: Chronic administration of formic acid has not been shown to cause significant adverse effects in laboratory animals. Malorny (1969a) administered 0.2% calcium formate in the drinking water to rats (Wistar) for 3 years, or 0.4% calcium formate for 2 years, and no adverse effect on growth, fertility, or function in up to 5 generations was reported. Other shorter-term studies were cited by Guest et al (1982), in which the only effect reported was a decrease in the rate of body weight gain in rats. No chronic studies in non-human primates are available. The occupational standard recommended by ACGIH (1985b) and adopted by OSHA for exposure to formic acid is 5 ppm (9 mg/m^3), a level designated to protect workers from developing adverse health effects attributable to irritation of the eyes, respiratory tract, and skin.

Potential Reproductive, Teratogenic, Mutagenic, and Carcinogenic Effects of Formic Acid Exposure: Injection of 5, 10, or 20 mg of sodium formate into fertilized chicken eggs did not produce toxic or teratogenic effects (Malorny 1969a). In a 5-generation study that used Wistar rats given 150 to 200 mg of calcium formate per day (Malorny 1969a), no adverse effect on reproduction was noted.

Formic acid has been reported to be mutagenic in Escherichia coli and in Drosophila germ cells but no effect on DNA transformation in Bacillus subtilis was found (Guest et al, 1982). No carcinogenicity studies have been reported for formic acid. In the chronic study by Malorny (1969a), in which Wistar rats were administered calcium formate for 2 or 3 years at a level of 0.4% or 0.2% in the drinking water, no gross tumor formation was reported; tumor formation, however, may not have been an endpoint that was specifically evaluated.

In summary, there is substantial evidence to suggest that formic acid (formate) is the causative agent in methanol-induced metabolic acidosis and ocular toxicity. Formic acid is a primary irritant upon direct contact to the skin, eye, or respiratory mucosa. Systemically, formic acid has been shown to cause few adverse effects either after acute or chronic administration. It did not cause reproductive or teratogenic effects in fertilized chicken eggs or rats. The carcinogenic potential of formic acid has not been evaluated. It should be noted, however, that it was found to be mutagenic in some microbial test systems. The 1976 FASEB report concluded that exposure from the use of formic acid or sodium formate as a food additive posed no threat to human health. Thus, it appears that the formate-associated effects on the visual system following acute high-level exposure to methanol are the effects of greatest concern with respect to formic acid.

APPENDIX VII

Reprinted from: Tephly, T.R. and McMartin, K.E. Methanol Metabolism and Toxicity, in Aspartame: Physiology and Biochemistry, Stegink, L.D. and Filer, L.J., Jr., eds., Marcel Dekker, New York, 1984, pp. 111-140, by courtesy of Marcel Dekker, Inc.

Metabolism of Methanol

Two enzymes are important in the oxidation of methanol to formaldehyde, alcohol dehydrogenase, and catalase (Fig. 2). The existence of relatively selective inhibitors for each enzyme has made it possible to test their importance in methanol oxidation in animals. It had been known for many years that the metabolism of methanol was blocked by the administration of ethanol and that methanol toxicity was attenuated by ethanol. Roe (8) suggested that humans who had taken ethanol simultaneously with methanol had less severe toxicity than when methanol was ingested alone. The assumption had existed for years that alcohol dehydrogenase was the major enzyme involved in methanol oxidation. Studies on alcohol dehydrogenase by Lutwak-Mann (52) showed that a partially

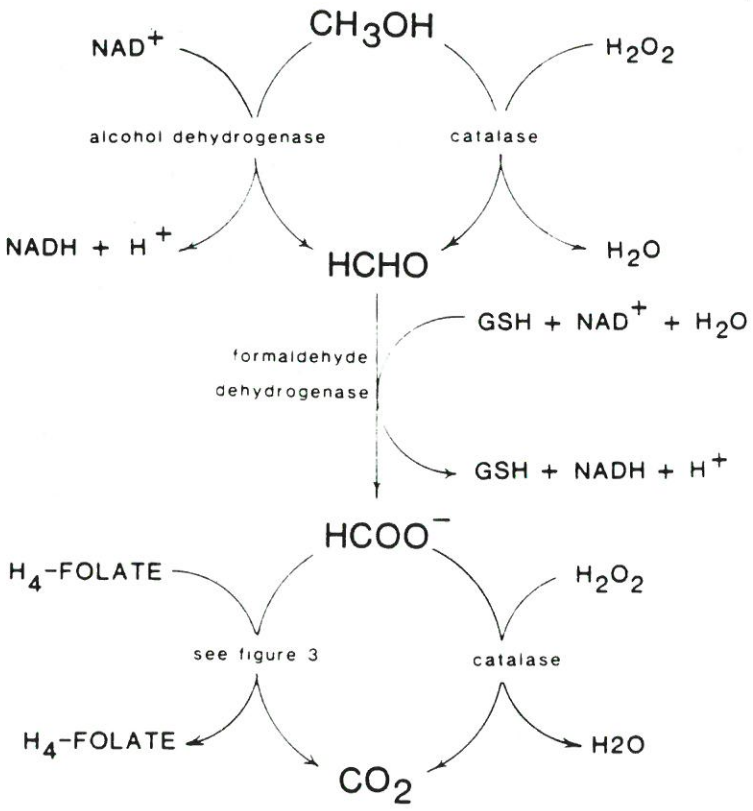


Figure 2 Biochemical reactions in the oxidation of methanol to carbon dioxide.

purified preparation of horse liver alcohol dehydrogenase oxidized methanol, although at a slower rate than ethanol. However, when crystalline horse liver alcohol dehydrogenase was prepared, it appeared to be incapable of catalyzing the oxidation of methanol (53,54), an observation that directed the attention of investigators to the catalase-peroxidative system as a mediator of the metabolism of methanol.

Interest returned to alcohol dehydrogenase and its role in methanol oxidation when Kini and Cooper (55) showed that, at high substrate concentrations, methanol was metabolized by crystalline horse liver alcohol dehydrogenase. Kini and Cooper (55) also showed that it was possible to copurify ethanol and methanol dehydrogenase activities from monkey liver. Their results conclusively demonstrated that alcohol dehydrogenase from monkey liver was capable of catalyzing methanol oxidation in vitro. Makar and Tephly (56) repeated these studies and showed that monkey liver alcohol dehydrogenase catalyzes methanol oxidation in vitro and that this activity is inhibited by the alcohol dehydrogenase

inhibitors pyrazole and 4-methylpyrazole. They reported that the Michaelis constant for methanol was about six times higher than that observed for ethanol, results similar to those found by Kini and Cooper, who had reported a K_m of 17 mM for methanol and 2.7 mM for ethanol. Makar and Tephly (56) observed K_m values of 20 mM for methanol and 3.2 mM for ethanol with the monkey liver enzyme. Pyrazole and 4-methylpyrazole were found to be competitive inhibitors when methanol and ethanol were utilized as substrates for monkey liver alcohol dehydrogenase. 4-Methylpyrazole yielded a K_i value of 9 μM which was about one-fourth that observed for pyrazole. Makar and Tephly (56) also showed that 4-methylpyrazole had no inhibitory properties toward catalase activity of rat liver homogenates in vitro or in vivo. Pyrazole, on the other hand, inhibits hepatic catalase activity when injected in vivo (57). Other studies have shown that purified hepatic alcohol dehydrogenase from rats (58) and humans (59,60) catalyze methanol oxidation. Although the Michaelis constant of methanol for alcohol dehydrogenase appears to be relatively high (10-100 mM), concentrations of this magnitude (20-30 mM) can be achieved in vivo after drinking a sizable quantity of either methanol or ethanol.

The inhibition of methanol oxidation by ethanol does not necessarily mean that the alcohol dehydrogenase system functions for methanol oxidation in a given animal species. Catalase can mediate the oxidation of a variety of alcohols to their corresponding aldehydes in the presence of a hydrogen peroxide-generating source (61). A study performed by Keilin and Hartree (62), using purified catalase and various peroxide-generating systems, showed that methanol and ethanol were metabolized at similar rates. Both rates were more rapid than those obtained with alcohols possessing higher molecular weights. Thus methanol and ethanol had about equivalent reactivities with the catalase peroxidative systems, whereas propanol and butanol appeared to display lower substrate reactivity. In fact, Keilin and Hartree suggested (62) that the physiological function of catalase might be involved with the metabolism of certain alcohols. Previously, it had been presumed that the exclusive function of catalase in the living organism was to decompose hydrogen peroxide. An important understanding of how alcohols such as ethanol or methanol might react with catalase in the presence of hydrogen peroxide was provided by Chance (63). He showed that substrates for catalase peroxide (complex I) react with substrates such as methanol and ethanol and promote the decomposition of the catalase peroxide complex, the rate of which was dependent upon the rate of reactivity with the substrate and the catalase-hydrogen peroxide complex. Chance postulated that catalase could conceivably account for most of the metabolism of methanol in the animal organism in vivo (63).

Definitive studies on methanol oxidation in vivo began with the use of selective inhibitors. Heim et al. (64) discovered that the herbicide 3-amino-1,2,4-triazole could inhibit hepatic and renal catalase activity in rats when injected intraperitoneally. This provided a means to test the direct participation of hepatic

catalase in the oxidation of methanol in vivo. Aminotriazole has since been a very useful and effective substance for studying the role of hepatic or renal catalase in the oxidation of agents in vivo. It does not inhibit erythrocyte catalase activity, nor does it affect liver cytochrome c content, blood hemoglobin levels, or urobilinogen excretion (64).

Nelson et al. (65) showed that aminotriazole had no effect on ethanol elimination in the dog, although hepatic catalase activity was markedly reduced. Mannering and Parks (66) showed that aminotriazole inhibited hepatic catalase activity in rats in vivo and that, in livers from rats whose hepatic catalase activity had been reduced by 90%, a marked inhibition of methanol oxidation to formaldehyde was observed in vitro. When crystalline beef liver catalase was added to reaction mixtures employing homogenates of rat liver obtained from aminotriazole-treated animals, methanol-oxidizing capacity was restored to control values. These results indicated that hepatic catalase activity was important for methanol oxidation in vitro and, furthermore, that the rate-limiting step in the process was likely to be the capacity of the liver to generate hydrogen peroxide (66). Thus, when peroxide-generating systems were added to hepatic homogenates in addition to crystalline liver catalase, a marked stimulation of activity beyond control values was observed. Mannering and Parks (66) also employed aminotriazole in order to determine whether catalase participated in the metabolism of methanol by rats in vivo. However, they found that aminotriazole had no effect on the rate of disappearance of methanol from the blood of rats. This apparent discrepancy was later explained (67) on the basis that considerable amounts of methanol are eliminated via excretory routes, as well as by metabolism, at the high doses of methanol which were employed in their studies (3 g/kg). When [^{14}C]methanol oxidation was studied by measuring $^{14}CO_2$ formation in vivo in rats, aminotriazole treatment markedly inhibited the oxidation of methanol to CO_2 (67).

Further evidence for a catalase-peroxidative system functioning in the metabolism of methanol in rats was provided in studies where ethanol and 1-butanol were employed as alternate substrate inhibitors of methanol oxidation. Ethanol and methanol have about equal reactivities with catalase peroxide complex I, while ethanol is 6-10 times more reactive than methanol with alcohol dehydrogenase (68). Thus if catalase was functioning in the oxidation of methanol by the rat, one would have expected a 50% inhibition of methanol oxidation by ethanol, and, if alcohol dehydrogenase were functionary, a 90% inhibition would have been expected. Tephly et al. (67) showed that when equimolar doses of ethanol and methanol were injected into rats, a 50% decrease in the rate of methanol oxidation occurred. When 1-butanol, which has only a slight reactivity with the catalase-hydrogen peroxide complex I, was injected, only a very slight inhibitory effect on methanol oxidation in the rat occurred. These results are consistent with the concept that the catalase-peroxidative system is the major catalyst of methanol oxidation in rats. Similar conclusions have been reached in isolated perfused rat liver experiments (69).

Although the role of a catalase-peroxidative system for the metabolism of methanol in rats was clear, different results were obtained with monkeys. Makar et al. (70) showed that pretreatment of monkeys with 1 or 3 g/kg body weight of aminotriazole 1 hr prior to methanol injection did not inhibit the rate of methanol metabolism, although hepatic catalase activity in livers from monkeys was reduced to 10% of control values. Studies were also performed using substrate inhibitors. When equimolar doses of ethanol and methanol were injected in monkeys, an 80% inhibition of the rate of methanol oxidation was observed (70). When 1-butanol, which produced only a slight effect on methanol oxidation in the rat, was injected into monkeys along with [^{14}C]methanol, a 90% inhibition of methanol oxidation was observed. Butanol is a highly reactive substrate for alcohol dehydrogenase, and, if alcohol dehydrogenase were functioning, one would have expected a 90% inhibition of methanol oxidation by 1-butanol. These results support the concept that the catalase-peroxidative system is not functional in methanol oxidation in the primate and that the metabolism of methanol in the monkey is dependent on the activity of alcohol dehydrogenase.

Other evidence for a major role of alcohol dehydrogenase in methanol oxidation in the monkey was provided by Watkins et al. (42), who showed that pyrazole markedly inhibited methanol oxidation in the rhesus monkey. Although pyrazole rapidly inhibited methanol metabolism in vivo, there was a possibility that inhibition of hepatic catalase activity by a pyrazole metabolite could be responsible for the inhibition of methanol oxidation in the monkey. Thus 4-methylpyrazole, a more potent inhibitor of alcohol dehydrogenase activity than pyrazole and one which does not inhibit hepatic catalase activity (56), was tested in the monkey (34). 4-Methylpyrazole was found to be a potent inhibitor of methanol oxidation with little or no effect on hepatic catalase activity.

Thus a major role of alcohol dehydrogenase in the metabolism of methanol in vivo in the monkey has been established. McMartin et al. (34) also showed that 4-methylpyrazole prevents the development of methanol poisoning in the monkey.

The question of why the peroxidative system does not function in the monkey has been examined. It should be recalled that Mannering and Parks (66) showed that when a peroxide-generating system was added to rat hepatic homogenates, peroxide generation appeared to be a rate-limiting factor. When a glucose and glucose oxidase preparation was added, marked stimulation of methanol oxidation occurred. When catalase activity had been reduced markedly, such as from aminotriazole-treated rats, glucose and glucose oxidase addition did not stimulate methanol oxidation (66). Goodman and Tephly (71) suggested that the monkeys may not metabolize methanol through a catalase-dependent system owing to decreased activity levels of peroxide-generating enzymes. Since peroxide-generating systems appear to be rate limiting for methanol oxidation via a catalase-dependent system in the rat, these workers proposed that this system should be rate limiting in the monkey, perhaps to an even greater degree (71) than noted in the rat. It is well known that urate oxidase activity is essentially absent in human liver, and

Goodman and Tephly (71) have shown that urate oxidase activity was also very low in monkey liver. Furthermore, glycolate oxidase activity, xanthine oxidase activity, and other peroxide-generating enzyme activities are also very low in monkey and human liver (72). This could account for why methanol oxidation in the monkey via a catalase-peroxidative system is difficult to demonstrate. Makar and Mannering (58) also suggested that the catalase distribution in the cell may be a consideration.

A third possible mechanism by which methanol could be oxidized to formaldehyde has been suggested by Rietbrock et al. (73) and Teschke et al. (74). This system, the hepatic microsomal mixed-function oxidase system, employs the hepatic endoplasmic reticulum, NADPH, and molecular oxygen.

METABOLISM OF FORMALDEHYDE

Formic acid was considered as the toxic agent in the acidosis seen in methanol poisoning until Van Slyke and Palmer (11) discredited the toxic role of formate. They failed to account for the increased organic acid excretion observed in methanol toxicity as due to formate. Potts (31) also failed to account for the organic acids excreted in the urine as due to formate following methanol poisoning in monkeys. Thus formaldehyde became a candidate as a causative agent in the toxicity of methanol poisoning (41,75,76), even though no one had demonstrated the presence of elevated formaldehyde levels in body fluids or tissues following methanol administration. Keeser (77) appeared to demonstrate the presence of formaldehyde in the cerebrospinal fluid, vitreous humor, and peritoneal fluid of rabbits which had been administered methanol. However, these studies were rather incomplete, lacked appropriate controls, and the method employed to measure formaldehyde lacked sensitivity and specificity. No formaldehyde could be detected in blood, urine, or tissues obtained from methanol-intoxicated animals in studies performed by Koivusalo (51) and Scott et al. (30) or from methanol-poisoned humans (8,78).

There are several ways by which formaldehyde can be disposed of in biological systems. First, formaldehyde has a high degree of reactivity with proteins and other endogenous compounds containing active hydrogen atoms (79). Formaldehyde can combine with any number of functional groups found in proteins or nucleic acids. Thus it may immediately form adducts with cellular constituents, leading to the formation of stable intermediates.

Strittmatter and Ball (80) isolated a formaldehyde-specific, NAD-dependent formaldehyde dehydrogenase from beef liver in 1955 and pointed out that this enzyme required reduced glutathione (GSH). This enzyme, which appears to be quite specific for formaldehyde, is often isolated with glutathionine thiolase (81, 82). In the reactions catalyzed by this enzyme (Fig. 2), formaldehyde combines with reduced GSH to form S-formyl glutathione, and in the presence of the thiolase, the product hydrolyzes to form formic acid and reduced glutathione. Reduced glutathione is therefore a key agent in the generation of formate from

formaldehyde. The first reaction appears to be freely reversible, but the second reaction is not, a feature which explains the apparent irreversibility of the two-step reaction as described by Strittmatter and Ball (80). Formaldehyde dehydrogenase activity is present in rat liver, human brain, and a number of other species and tissues such as retina (83). These tissues have not been examined adequately for the presence of S-formyl glutathione hydrolase. The specific activity of this enzyme in crude preparations appears to be quite high, and its presence would be expected in other tissues (81).

Formaldehyde oxidation can also occur in liver mitochondria through an aldehyde dehydrogenase activity (or activities) which is likely to be similar to the aldehyde dehydrogenases of mitochondria that have been described previously (84-86). Aldehyde dehydrogenase activity of mitochondria appears to be very high and is capable of reacting nonspecifically with many aldehyde substrates. Thus it is likely that formaldehyde-oxidizing capabilities of liver are extremely high, either through the formaldehyde dehydrogenase-S-formyl glutathione hydrolase system or through aldehyde dehydrogenase activities in mitochondria or cytosol. Goodman and Tephly (87) have shown that the formaldehyde dehydrogenase activity of human liver is, in fact, higher than that of rat liver. Thus one cannot explain, at this time, the fact that methanol poisoning is uniquely present in humans or monkeys on the basis of an inability to metabolize formaldehyde, since the conversion of formaldehyde to formate can apparently proceed as readily in humans as it does in rats.

Formaldehyde can be metabolized through the tetrahydrofolic acid-dependent one-carbon pool which is capable of utilizing one-carbon units at various oxidation levels and transferring these one-carbon moieties to various endogenous acceptors. Apparently, free formaldehyde enters these reactions by combining with tetrahydrofolate nonenzymatically (88) or through the formaldehyde-activating enzyme to form N^5,N^{10}-methylenetetrahydrofolate. This enzyme has been demonstrated in pigeon liver by Osborn et al. (89) and has been found to be present in a number of mammalian tissues (90).

The metabolism of formaldehyde has been studied by Malorny et al. (91) in dogs and cats in vivo. These investigators administered formaldehyde intravenously and orally to dogs and showed that there was a rapid appearance of formic acid in blood plasma and the presence of only negligible levels of formaldehyde in blood. Experiments in vitro with human blood showed that formaldehyde was oxidized to formic acid (92,93). Rietbrock (94) showed that in dogs, cats, rabbits, guinea pigs, and rats the infusion of formaldehyde resulted in a rapid disappearance of formaldehyde from the blood with a half-life of approximately 1 min. Malorny et al. (91) found that when equimolar amounts of formaldehyde, formic acid, or sodium formate were infused in dogs, the peak concentrations of formic acid in the plasma were equivalent in all three cases, indicating that formaldehyde was rapidly metabolized to formic acid.

Although it is possible that formaldehyde may be responsible for certain of

the toxic findings in methanol poisoning, it would be unlikely that it could account for the metabolic acidosis, since formate appears to be the major factor in the metabolic acidosis seen in monkeys and humans poisoned with methanol. It is also unlikely that formaldehyde can be generated in the liver and delivered to the optic nerve in an intact state. Therefore either formaldehyde forms a product with some endogenous acceptor which is responsible for the ocular toxicity, or formaldehyde is generated in situ in the eye, where it may exert an effect on the ocular system. Although these possibilities cannot be ruled out at this time, the responsibility of formaldehyde for the ocular toxicity of methanol is unlikely, since formate itself can produce ocular toxicity in the monkey (95). In studies where blindness in monkeys was produced from formate, no formaldehyde could be detected in body fluids or tissues (95). In any case, more studies need to be performed on the fate of formaldehyde in the organism in order to disregard it as a toxic agent in the methanol poisoning syndrome in man.

FORMATE METABOLISM

Nonprimates

The ability of animal tissues to oxidize formate into CO_2 was first reported by Batelli (96) and Battelli and Stern (97), who observed that tissues obtained from a variety of animals, such as the horse, cow, sheep, dog, and rabbit, were capable of oxidizing formate into CO_2 in the presence of hydrogen peroxide (Fig. 2). More that 40 years later, Chance (63) studied the kinetics of the catalase-hydrogen peroxide system with different substrates and showed that formate reacts with the hydrogen peroxide catalase complex (complex I).

In subsequent years, a number of in vitro investigations strongly indicated a key role of the catalase-hydrogen peroxide system in the oxidation of formate. Some of the experimental results leading to this conclusion are the following:

1. There is a good correlation between the formate-oxidizing ability and the catalase activity in liver preparations of different species (98), in different tissues within one animal species (99), and in the subcellular compartments from tissue preparations (100).
2. Administration of aminotriazole to guinea pigs greatly lowered the formate-oxidizing ability of liver fractions in vitro (98).
3. Certain types of neoplasms in rats (101), mice (102), and humans (103) lead to a marked lowering of both catalase activity and the formate-oxidizing ability in vitro.
4. Folate-deficient rats possess a marked impairment in formate-oxidizing ability (104) and lowered hepatic catalase levels.
5. Decreased formate oxidation in vitro results from decreased hydrogen peroxide generation caused by factors such as a decreased hepatic xanthine oxidase activity, vitamin B_6 deficiency (105), or thyrotoxicosis (100). On the

other hand, factors that increase hydrogen peroxide generation stimulate formate oxidation. This can be accomplished by supplementing liver preparations with hypoxanthine, a known substrate of xanthine oxidase.

Another path of formate oxidation to CO_2 is the folate biochemical pathway (88,90,106,107). Formate enters into the folate pool by combining with tetrahydrofolate (THF) to form 10-formyl-THF, a reaction catalyzed by 10-formyl-THF synthetase, an enzyme widely distributed among mammalian tissues (108). Kutzbach and Stokstad (109) showed that 10-formyl-THF oxidoreductase catalyzes the oxidation of the formyl group directly to CO_2. Thus there is a two-step conversion of formate to CO_2.

Rietbrock et al. (73) suggested that exogenously administered formate, or formate arising from methanol metabolism in vivo, is oxidized via the folate-dependent pathway. They found an inverse correlation between plasma levels of folate in different animal species and the half-life of exogenously administered formate. They also reported that dogs accumulated formic acid to a small extent (2 mEq/liter) in their blood following methanol administration. Pretreatment of dogs with folic acid prior to methanol produced a lower blood formate level, whereas methotrexate (an inhibitor of dihydrofolate reductase) had the opposite effect (110).

Palese and Tephly (111) measured $^{14}CO_2$ formation following [^{14}C] formate administration to rats and showed that folate deficiency resulted in a greatly diminished rate of formate oxidation. In contrast, administration of aminotriazole, the potent catalase inhibitor, did not inhibit the rate of formate oxidation to CO_2. Administration of ethanol in molar ratio of 22:1 (ethanol:formate) did not alter the rate of formate oxidation in the rat. However, in folate-deficient rats, the catalase-hydrogen peroxide system may serve as an alternate pathway, since, in folate-deficient rats aminotriazole or ethanol administration did result in some inhibition of the rate of formate oxidation (111).

The knowledge that formate is being metabolized in vivo via a folate-dependent system has been utilized to advantage in order to produce metabolic acidosis in rats after methanol treatment. Rats, made folate deficient, oxidize formate at a markedly slowed rate (111,112), and administration of methanol (4 g/kg) to folate-deficient rats leads to high formate levels and severe metabolic acidosis (113). Blood formate levels reached values as high as 18 mEq/liter in these animals. This value is higher than blood formate levels noted in methanol-poisoned monkeys (34).

Monkeys

In monkeys the folate-dependent pathway is also the major route of formate oxidation to CO_2. Makar et al. (70) showed that aminotriazole had no effect on methanol oxidation to CO_2 in the monkey, and McMartin et al. (114) demonstrated that neither the rate of formate oxidation nor the half-life of formate in

the blood was altered by aminotriazole. However, the rate of formate metabolism in folate-deficient monkeys was approximately 50% lower than that observed in control monkeys. Formate oxidation was stimulated in monkeys by the administration of either folic acid (114) or 5-formyl-THF (115).

McMartin et al. (114) also showed that the sensitivity of monkeys to methanol was related to folate, since folate-deficient monkeys became especially sensitive to the toxicity of methanol relative to the amounts of formate produced. Thus, when 0.5 g/kg of methanol was given to either folate-deficient or control monkeys, the level of blood formate in the folate-deficient animals was more than two times greater than that observed in the control animals.

Noker and Tephly (115) then showed that methanol toxicity can be modified considerably in monkeys by the administration of folate derivatives. These workers followed the course of methanol toxicity in monkeys administered [^{14}C] methanol (2 g/kg) or [^{14}C] methanol with repetitive doses of 5-formyl-THF. In monkeys treated with 5-formyl-THF (2 mg/kg at 0, 4, 8, 12, and 18 hr after methanol), blood formate levels were significantly decreased (by at least 50%) from those observed in the untreated animals. Similar results were obtained when sodium folate was employed instead of 5-formyl-THF. In both treated and untreated monkeys, the elimination of methanol from blood followed zero-order kinetics and proceeded at a rate of 7.9 mg/dl per hour in the 5-formyl-THF-treated animals, and at 7.1 mg/dl per hour in the untreated animals. Therefore the clearance of methanol was not altered by folate administration. In addition, the distribution and route of metabolism of [^{14}C] methanol did not appear to be changed by 5-formyl-THF treatment, since the total amount of ^{14}C label recovered in urine as either expired [^{14}C] methanol or $^{14}CO_2$ was the same for both treated and untreated monkeys. However, the rate of methanol oxidation to CO_2 was significantly increased in those animals treated with 5-formyl-THF, and folate treatment was effective in reducing blood formate levels by increasing the rate of formate metabolism to CO_2. Blood pH and blood bicarbonate levels remained within the normal range in animals treated with 5-formyl-THF, in contrast to the marked bicarbonate depletion, high blood formate levels, and metabolic acidosis observed in animals not given 5-formyl-THF.

Noker and Tephly (115,116) have also shown that 5-formyl-THF (when given in repetitive doses) is effective in reversing methanol toxicity in the monkey once it has developed. The accumulation of blood formate in monkeys could be markedly altered by 5-formyl-THF, even when administered after toxicity became apparent. A rapid decline in blood formate levels was observed in methanol-poisoned animals several hours after the initiation of 5-formyl-THF treatment. In monkeys not given 5-formyl-THF, formate levels continued to climb. The decline in formate concentrations in monkeys treated with folate was coupled to an increase in the rate of CO_2 formation from methanol.

The results demonstrate that the severity of methanol toxicity in monkeys is correlated with accumulation of formate in the blood and that this can be sig-

nificantly modified by procedures which provide the monkey with more folate. These results suggest that there is a reciprocal relationship between the formate oxidation rate and the hepatic folate level of the animal. They suggest the possible use of folates for the treatment of human methanol toxicity.

Regulation of Formate Oxidation Through Regulation of Tetrahydrofolate

Since the folate biochemical pathway is primarily involved in the metabolism of formate, the regulation of the rate of formate metabolism is governed by the regulation of the hepatic tetrahydrofolate concentrations in liver. This concept has been advanced recently by studies which have explored the role of 5-methyl-THF:homocysteine transmethylase (methionine synthetase). This cytosolic enzyme is reponsible for the methylation of homocysteine to form methionine as well as for the conversion of 5-methyl-THF to THF (Fig. 3). It requires methyl-

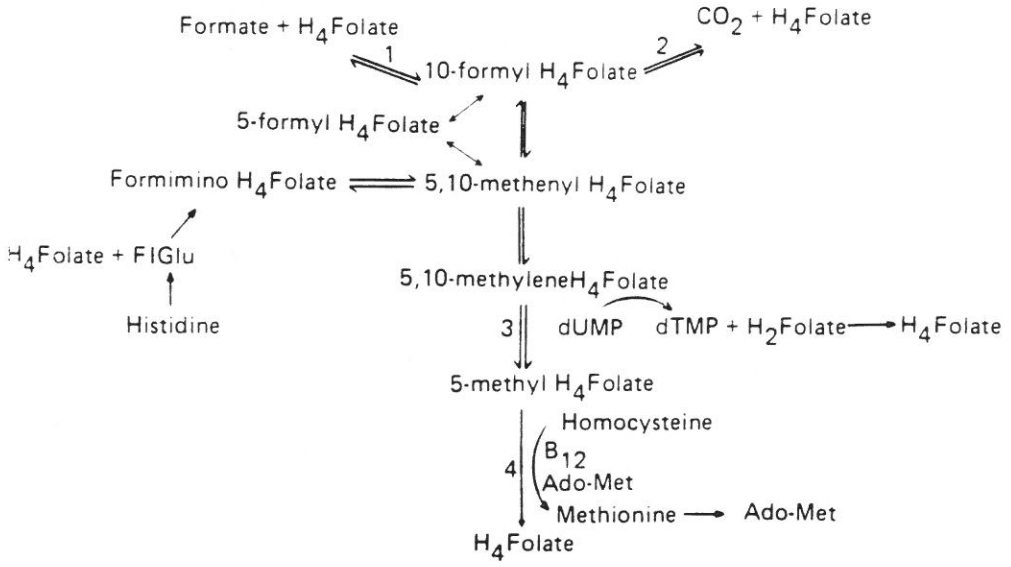


Figure 3 Pathway of folate-dependent formate metabolism (H_2 folate, dihydrofolate; H_4 folate, tetrahydrofolate; B_{12}, vitamin B_{12}; Ado-Met, S-adenosylmethionine). Reaction 1 is catalyzed by formyl-tetrahydrofolate synthetase and requires activation of formate by ATP. Reaction 2 is catalyzed by formyl-tetrahydrofolate dehydrogenase and utilizes NADP+. Reaction 3 is catalyzed by methylene-tetrahydrofolate reductase and is thought to be irreversible. Reaction 4 is catalyzed by methyl-tetrahydrofolate homocysteine methyltransferase (methionine synthetase) and is dependent upon vitamin B_{12} and catalytic amounts of adenosylmethionine, a reducing system.

cobalamin and S-adenosylmethionine for maximal activity. As far as we know, methionine synthetase is the only methlycobalamin-dependent enzyme in the mammalian organism. The anesthetic gas nitrous oxide has been reported to react with transition methyl complexes, such as the cobalt-ligand complex in vitamin B_{12}, and oxidizes the coenzyme from the active cob(I)alamin form to the inactive cob(III)alamin form (117). Deacon et al. (118) have shown the inhibition of hepatic and brain methionine synthetase activity in vivo by nitrous oxide, and Eells et al. (119,120) demonstrated that, following nitrous oxide treatment of rats, there was a significant decrease in hepatic levels of tetrahydrofolate forms and an increase in hepatic 5-methyl-THF. Rats treated with nitrous oxide also exhibited a marked decrease in the rate of formate oxidation to carbon dioxide. When methanol (4 g/kg) was administered to rats which were exposed to nitrous oxide:oxygen (50:50) for 2 hr, there was a marked metabolic acidosis in these animals, with accumulation of blood formate, a decrease in blood pH to 7.2, and a depletion of blood bicarbonate. This metabolic acidosis produced after the administration of methanol to rats had not been demonstrated previously, except where rats were made folate deficient (104). Hepatic methionine synthetase activity was reduced to 10% of control levels in animals treated with N_2O:O_2 (50:50), a finding which accounts for the depletion of hepatic tetrahydrofolate. Recently, Eells et al. (120) demonstrated an excellent correlation between the rate of formate oxidation in rats with hepatic tetrahydrofolate levels. Since S-adenosylmethionine levels are also dependent upon hepatic methionine levels, one would expect alteration of S-adenosylmethionine concentrations in liver. S-Adenosylmethionine levels are depleted by the treatment of rats with nitrous oxide, and a good correlation between tetrahydrofolate levels and S-adenosylmethionine was also recorded (120).

Methionine administration to rats which have been treated with nitrous oxide leads to a reversal of the depletion of tetrahydrofolate levels in liver and a reversal of the inhibition of formate oxidation produced by nitrous oxide (120). However, the mechanism by which methionine is capable of reversing the depletion of tetrahydrofolate brought on by nitrous oxide treatment is still unexplained; that is, although nitrous oxide inhibits methionine synthetase activity and depletes tetrahydrofolate levels, methionine administration does not reverse the inhibition of methionine synthetase activity, although it restores tetrahydrofolate in liver. Therefore methionine cannot be exerting its effect by a direct action on methionine synthetase activity. It is possible that methionine exerts its effect through the elevation of S-adenosylmethionine concentrations in liver. Following methionine treatment, there is a marked elevation of S-adenosylmethionine levels in rat liver (120) and S-adenosylmethionine acts as an inhibitor of 5,10-methylene-THF reductase (121). More work is needed in order to determine the mechanism by which methionine exerts its reversal of the nitrous oxide depletion of hepatic tetrahydrofolate.

Recent studies in our laboratory have shown that treatment of monkeys with a nitrous oxide:oxygen (50:50) mixture leads to marked sensitization of the monkey to methanol toxicity. Following a dose of 1 g/kg of methanol (a dose which produces only a slight increase in blood formate in monkeys), there was a marked accumulation of formate (4 mEq/liter) 12 hr after methanol. These values are greater than blood formate levels observed when 2 g/kg of methanol were given to air-breathing monkeys.

A great deal more work is needed in order to understand which step of the many enzymatic reactions in the folate biochemical pathway regulates the regeneration of tetrahydrofolate in monkeys. However, it is important to realize that primates are at some risk with respect to their folate regulation; and it would appear to be important for future work to determine that step or process which is deficient and which places the primate at a distinct liability when it comes to the disposition of one-carbon moieties.

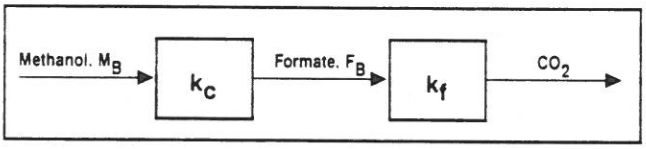
APPENDIX VIII

KINETIC MODEL OF FORMATE ACCUMULATION FOLLOWING ACUTE METHANOL EXPOSURE

The following two-compartment model may be used to estimate blood formate levels following brief (less than 15 minutes), low-level exposures to methanol. Since blood clearance of methanol proceeds with about a 3-hour half-time, such brief exposures can be considered as a a single acute dose. The model assumes:

1. All metabolic pathways remain in a first-order domain.
2. Formate distributes relatively evenly to body water, and renal excretion of formate is negligible compared to metabolism.
3. The entire dose of methanol enters the metabolic pathway.

Consider the following system:



Let

t = time post-ingestion (hours)

M_B = aqueous concentration of blood methanol (mM, i.e., millimoles per liter) and, $M_B = M_{Bo}$ at $t = 0$.

F_B = aqueous concentration of blood formate (mM)

k_c = clearance rate constant of methanol into the metabolic pathway

k_f = rate constant of formate metabolism

Then, using first-order principles,

(1) $\frac{dF_B}{dt} = \frac{-dM_B}{dt} - k_f F_B$ and since, (2) $M_B = M_{Bo}e^{-k_c t}$,

(3) $\frac{dM_B}{dt} = -k_c M_{Bo} e^{-k_c t}$

Therefore,

(4) $\frac{dF_B}{dt} + k_f F_B = k_c M_{Bo} e^{-k_c t}$

Multiplying both sides by $e^{k_f t}$ yields

(5) $e^{k_f t}\frac{dF_B}{dt} + k_f F_B e^{k_f t} = k_c M_{Bo} e^{(k_f - k_c)t}$

OR

(6) $\frac{d(F_B e^{k_f t})}{dt} = k_c M_{Bo} e^{(k_f - k_c)t}$

Integrating and setting $F_B = 0$ at $t = 0$ yields

(7) $F_B = \frac{k_c}{k_f - k_c} M_{Bo} (e^{-k_c t} - e^{-k_f t})$

and, setting dF_B/dt equal to zero solves for the time, t_{max}, at which F_B is maximized,

(8) $t_{max} = \frac{\ln (k_c/k_f)}{k_c - k_f}$

Finally, the ratio of the maximized concentration of formate to the initial concentration of methanol is

(9) $\frac{F_{Bmax}}{M_{Bo}} = \frac{k_c}{k_f - k_c} (e^{-q} - e^{-r})$

where, $q = \frac{k_c}{k_c - k_f} \ln (k_c/k_f)$, and $r = \frac{k_f}{k_c - k_f} \ln (k_c/k_f)$

This value depends only on the ratio of k_f to k_c (as does the "dimensionless time" $k_c t_{max}$).

Thus, one may easily project blood formate levels using measured values of k_c and k_f, as in the following example:

A worst-case exposure in a hot-soak garage produces a methanol body burden of 1 mg/kg, which is equivalent to 0.05 mM (M_{Bo}). Methanol clears from the bloodstream into the metabolic pathway with a $T_{1/2}$ of 3 hours, meaning $k_c = 0.693/3 = 0.23\ hr^{-1}$, and formate clearance proceeds with a $T_{1/2}$ of 45 minutes or $k_f = 0.92\ hr^{-1}$.

Applying formulas (7) and (8) yields $t_{max} = 2.0$ hours, at which time $F_{Bmax} = 0.0082$ mM. Since measured levels of background formate are about 0.2 mM, the maximal increment of formate amounts to 4% of background. For the example described, Figure VII-1 displays the time courses that equations (2) and (7) predict for methanol and formate, respectively. Using the example's clearance characteristics as a point of reference, Table VII-1 (center box is reference) shows the relative values of t_{max} and F_B as $T_{1/2}$ for methanol and formate vary. The table shows the expected: as the efficiency of formate metabolism decreases relative to methanol clearance, formate accumulates to a greater degree. In addition, formate peaks, for all cases shown, within 1.3 to 2.7 hours of exposure. However, all the curve shapes remain similar to the one plotted for the example.

$T_{1/2}$ Formate (min) \ $T_{1/2}$ Methanol (hour)	2	3	4
30	1.00 / 0.67	0.74 / 0.78	0.59 / 0.86
45	1.32 / 0.85	Fmax 1.00 / 1.00 tmax	0.81 / 1.11
60	1.59 / 1.00	1.22 / 1.19	1.00 / 1.33

upper left = maximal formate concentration relative to base case
lower right = time to achieve maximal formate relative to base case

Table VIII-1 Relative values of (1) peak blood formate level and (2) the time from methanol exposure to achieve that level as a function of the blood clearance half-times for methanol and formate. In reference condition (middle cell), half-time for methanol clearance is 3 hours and for formate is 45 minutes. Upper left of each cell is relative blood formate level; lower right is relative time to achieve maximal concentration.

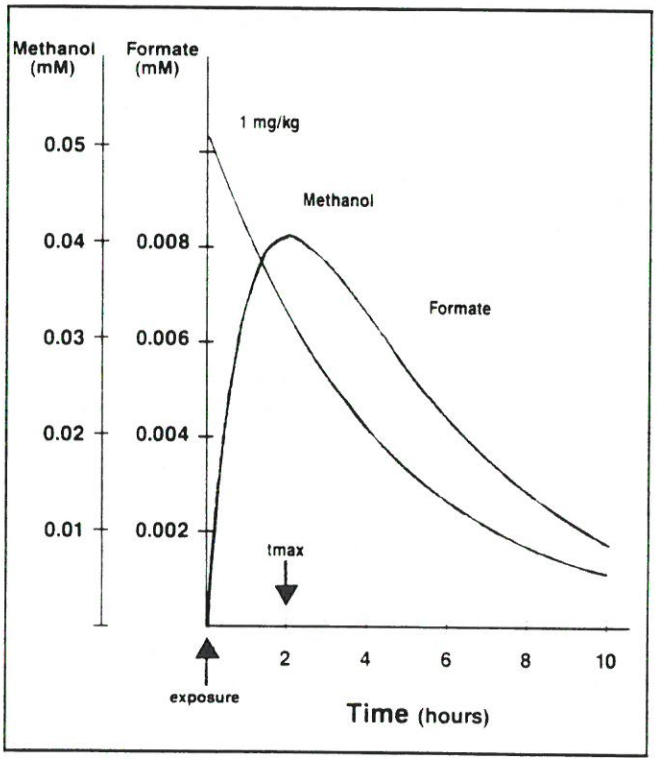


Figure VIII-1: Blood levels of methanol and formate following an initial body burden of 1 mg/kg methanol according to the two-compartment model presented in the text of Appendix VIII. Clearance half-times are 3 hours for methanol and 45 minutes for formate.

REFERENCES

Abbondandolo, A., Bonatti, S., Corsi, C., Corti, G., Fiorio, R., Leporini, C., Mazzaccaro, A., and Nieri, R. The use of organic solvents in mutagenicity testing. Mutat. Res. 79:141-150, 1980.

Andrews, L.S., Clary, J.J., Terrill, J.B., and Bolte, H.F. Subchronic inhalation toxicity of methanol. J. Toxicol. Environ. Health 20:117-124, 1987.

Aquilonius, S.M., Askmark, H., Enoksson, P., Lundberg, P.O., and Mostrum, U. Computerised tomography in severe methanol intoxication. Br. Med. J. ii: 929-930, 1978.

ARB, 1986. Air Resources Board, California Energy Commission, and South Coast Air Quality Management District. "Report of the Three-Agency Methanol Task Force." Volume I - Executive Summary, Volume II - Appendices. May 15, 1986.

ACGIH, 1985a. American Conference of Governmental Industrial Hygienists, Inc. (ACGIH). Documentation of the Threshold Limit Values and Biological Exposure Indices. Fifth edition. Cincinnati, OH, 1985, p.372.

ACGIH, 1985b. Ibid., p.279

Anderson, E. Methanol touted as best alternate fuel for gasoline. Chem. Engr. News, June 11, 1984.

Bartlett, G.R. Combustion of C^{14} labelled methanol in the intact rat and its isolated tissues. Am. J. Physiol. 163:614-621, 1950.

Baumann, K. and Angerer, J. Occupational chronic exposure to organic solvents. VI. Formic acid concentration in blood and urine as an indicator of methanol exposure. Int. Arch. Occup. Environ. Health 42:241-249, 1979.

Baumbach G.L., Cancilla, P.A., Martin-Amat, G., Tephly, T.R., McMartin, K.E., Makar, A.B., Hayreh, M.S., and Hayreh, S.S. Methyl alcohol poisoning. IV. Alterations of the morphological findings of the retina and optic nerve. Arch. Ophthalmol. 95:1859-1865, 1977.

Black, K.A., Eells, J.T., Noker, P.E., Hawtrey, C.A., and Tephly, T.R. Role of hepatic tetrahydrofolate in the species difference in methanol toxicity. Proc. Natl. Acad. Sci. 82:3854-3858, 1985.

Bennett, I.L., Jr., Cary, F.H., Mitchell, G.L., Jr., and Cooper, M.N. Acute methyl alcohol poisoning: A review based on experiences in an outbreak of 323 cases. Medicine 32:431-463, 1953.

Benton, C.D. and Calhoun, F.P. The ocular effects of methyl alcohol poisoning. Report of a catastrophe involving three hundred and twenty persons. Trans. Am. Acad. Ophth. 56:875-883, 1952.

Buller, F. and Wood, C.A. Poisoning by wood alcohol. Cases of death and blindness from Columbian spirits and other methylated preparations. J. Am. Med. Assoc. 43:1058-1062, 1117-1123, 1213-1221, 1289-1296, 1904.

Burk, M. On chronic occupational methyl alcohol intoxication Klin Monatabl Augenheilkd 130:845-850, 1957. (German)

Cameron, A.M., Nilsen, O.G., Haug, E., and Eik-Nes, K.B. Circulating concentrations of testosterone, luteinizing hormone and follicle stimulating hormone in male rats after inhalation of methanol. Arch. Toxicol., Suppl. 7:441-443, 1984.

Carey, P.M. Determination of a Range of Concern for Mobile Source Emissions of Formaldehyde Based Only on Its Toxicological Properties. U.S. Environmental Protection Agency, Office of Air Noise and Radiation, Office of Mobile Sources, Emission Control Technology Division, EPA-AA-TSS-83-5, July 1983.

Casarett and Doull, 1975. Toxicology: The Basic Science of Poisons. Casarett, L.J. and Doull, J., eds., Macmillan Publishing Co. Inc., New York, NY, 1975.

CEN, 1986. New York City to test methanol-fueled buses. Chem. Engr. News, June 30, 1986.

Chao, Chen-Tsi. Materials on the hygienic standardization of the maximally permissible concentration of methanol vapors in the atmosphere. Gig. Sanit. 24:7-12, 1959. (Russian)

Chew, W.B., Berger, E.H., Brines, O.A., and Capron, M.J. Alkali treatment of methyl alcohol poisoning. J. Am. Med. Assoc. 130:61-64, 1946.

Clay, K.L., Murphy, R.C., and Watkins, W.D. Experimental methanol toxicity in the primate: Analysis of metabolic acidosis. Toxicol. Appl. Pharmacol. 34:49-61, 1975.

De Schweinitz, G.E. A case of methyl-alcohol amaurosis, the pathway of entrance of the poison being the lungs and the cutaneous surface. Ophthalmic Rec. 10:289-296, 1901.

Dethlefs, R. and Naraqi, S. Ocular manifestations and complications of acute methyl alcohol intoxication. Med. J. Aust. 2:483-485, 1978.

Eells, J.T., Makar, A.B., Noker, P.E., and Tephly, T.R. Methanol poisoning and formate oxidation in nitrous oxide treated rats. J. Pharmacol. Exp. Ther. 217:57-61, 1981.

Eells, J.T., Black, K.A., Makar, A.B., Tedford, C.E., and Tephly T.R. The regulation of one-carbon oxidation in the rat by nitrous oxide and methionine. Arch. Biochem. Biophys. 219:316-326, 1982.

Eells, J.T., Black K.A., Tedford, C.E., and Tephly, T.R. Methanol toxicity in the monkey: Effects of nitrous oxide and methionine. J. Pharmacol. Exp. Ther. 227:349-353, 1983

Eriksen, S.P. and Kulkarni, A.B. Methanol in normal human breath. Science 141:639-640, 1963.

Erlanson, P., Fritz, H., Hagstam, K.E., Liljenberg, B., Tryding, N., and Voigt, G. Severe methanol intoxication. Acta Medica Scand. 177:393-408, 1965.

FASEB. 1976. Federation of American Societies for Experimental Biology. Evaluation of the Health Aspects of Formic Acid, Sodium Formate, and Ethyl Formate as Food Ingredients. Prepared for Food and Drug Administration, Washington, DC. Bureau of Foods. PB-266 282, 1976.

FPF. 1982. Report of the Federal Panel on Formaldehyde. Environ. Health Perspect. 43:139-168, 1982.

Francot, P. and Geoffroy, P. Le methanol dans les jus de fruits, les boissons, fermentees, les alcools et spiritueux. Rev. Ferment. Ind. Aliment. 11:279-287, 1956. (French)

Frederick, L.J., Schulte, P.A., and Apol, A. Investigation and control of occupational hazards associated with the use of spirit duplicators. Am. Ind. Hyg. Assoc. J. 45:51-55, 1984.

Gagnon, C. and Heisler, S. Protein carboxyl-methylation: Role in exocytosis and chemotaxis. Life Sciences 25:993-1000, 1979.

Gilger, A.P., Farkas, I.S., and Potts, A.M. Studies on the visual toxicity of methanol. X. Further observations on the ethanol therapy of acute methanol poisoning in monkeys. Am. J. Ophthalmol. 48:153-161, 1959.

Gilger, A.P. and Potts, A.M. Studies on the visual toxicity of methanol. V. The role of acidosis in experimental methanol poisoning. Am. J. Ophthalmol. 39:63-85, 1955.

Gilger, A.P., Potts, A.M., and Johnson, L.V. Studies on the visual toxicity of methanol. II. The effect of parenterally administered substances on the systemic toxicity of methyl alcohol. Am. J. Ophthalmol. 35:113-126, 1952.

Gilger, A.P., Potts, A.M., and Farkas, I.S. Studies on the visual toxicity of methanol. IX. The effect of ethanol on methanol poisoining in the rhesus monkey. Am. J. Ophthalmol. 42:244-252, 1956.

Gimenez, E.R., Vallejo, N.E., Roy, E., Lis, M., and Izurieta, E.M., Rossi, S., and Capuccio, M. Percutaneous alcohol intoxication. Clin. Toxicol. 1:39-48, 1968.

Gonda, A., Gault, H., Churchill, D., and Hollomby, D. Hemodialysis for methanol intoxication. Am. J. Med. 64:749-757, 1978.

Gold, M.D. Organic emission standards for light-duty methanol-fueled vehicles: A methodology. Presented at the 78th Annual Meeting of the Air Pollution Control Association, Detroit, MI, June 16-21, 1985.

Gray, C.L., Jr. and Alson, J.A. Moving America to Methanol, University of Michigan Press, Ann Arbor, MI, 1985.

Greenburg, L., Mayers, M.R., Goldwater, L.J., and Burke, W.J. Health hazards in the manufacture of "fused collars": II. Exposure to acetone-methanol. J. Ind. Hyg. Toxicol. 20:148-154, 1938.

Guest, D., Katz, G.V., and Astill, B.D. Aliphatic carboxylic acids. in Patty's Industrial Hygiene and Toxicology, Volume IIC, Clayton, G.D. and Clayton, F.E., eds., John Wiley and Sons, New York, NY, 1982, pp. 4901-4987.

Guggenheim, H.A., Couch, J.R., and Weinberg, W. Motor dysfunction as a permanent complication of methanol ingestion. Presentation of a case with a beneficial response to levodopa treatment. Arch. Neurol. 24:550-554, 1971.

Guyton, A.C. Textbook of Medical Physiology, Sixth Edition, W.B. Saunders Company, Philadelphia, PA, 1981, pp. 742-743.

Harger, R.N., Raney, B.B., Bridwell, E.G., and Kitchel, M.F. The partition ratio between air and water, urine and blood: estimation and identification of alcohol in these liquids from analysis of air equilibrated with them. J. Biol. Chem. 183:197-213, 1950.

Harvey, C.A. "Determination of a Range of Concern for Mobile Source Emissions of Methanol." U.S. Environmental Protection Agency, Office of Air, Noise and Radiation, Office of Mobile Sources, Emission Control Technology Division, EPA/AA/TSS/83-6, July 1983.

Harvey, C.A., Carey, P.M., Somers, J.H., and Garbe, R.J. Toxicologically acceptable levels of methanol and formaldehyde emissions from methanol-fueled vehicles. SAE Technical Paper Series, # 841357, Fuels and Lubricants Meeting and Exposition, Baltimore, MD, October, 8-11, 1984.

Hawes, A.T. Amblyopia from the fumes of wood alcohol. Boston Med. Sur. J. 153:525, 1905.

Hayreh, M.S., Hayreh, S.S., Baumbach, G.L., Cancilla, P., Martin-Amat, G., Tephly, T.R., McMartin, K.E., and Makar, A.B. Methyl alcohol poisoning. III. Ocular toxicity. Arch. Ophthalmol. 95:1851-1858, 1977.

Heinrich, R. and Angerer, J. Occupational chronic exposure to organic solvents. X. Biological monitoring parameters for methanol exposure. Int. Arch. Occup. Environ. Health 50:341-349, 1982.

Humperdinck, K. On the problem of chronic intoxication with methanol vapors. Arch Gewerbepathol Gerwergehyg 10:569-574, 1941. (German)

Infurna R. and Weiss B. Neonatal behavioral toxicity in rats following prenatal exposure to methanol. Teratology 33:259-265, 1986.

Ingalls, M.N., and Garbe, R.J. Ambient pollutant concentrations from mobile sources in microscale situations. SAE Technical Paper Series, #820787, Passenger Car Meeting, Troy, MI, June 7-10,1982.

Jacobsen, D., Ovrebo, S., Arnesen, E., and Paus, P.N. Pulmonary excretion of methanol in man. Scand. J. Clin. Lab. Invest. 43:377-379, 1983.

Jacobsen, D. and McMartin, K.E. Methanol and ethylene glycol poisonings. Mechanism of toxicity, clinical course, diagnosis and treatment. Medical Toxicology 1:309-334, 1986.

Jelliffe, S.E. Multiple neuritis in wood alcohol poisoning. Med. News 86:387-390, 1905.

Johlin, F.C. Jr., Fortman, C.S., Nghiem, D.D., and Tephly, T.R., Studies in human hepatic biochemistry (abs). Fed. Proc. 45:277, 1986.

Kane, R.L., Talbert, W., Harlan, J., Sizemore, G., and Cataland, S. A methanol poisoning outbreak in Kentucky. A clinical epidemiologic study. Arch. Environ. Health 17:119-129, 1968.

Kaplan, K. Methyl alcohol poisoning. Am. J. Med. Sci. 244:170-174, 1962.

Kingsley, W.H. and Hirsch, F.G. Toxicologic considerations in direct process spirit duplicating machines. Compen. Med. 40:7-8, 1954-1955.

Kini, M.M. and Cooper, J.R. Biochemistry of methanol poisoning. III. The enzymatic pathway for the conversion of methanol to formaldehyde. Biochem. Pharmacol. 8:207-215, 1961.

Koivusalo, M. Methanol. In International Encyclopedia of Pharmacology and Therapeutics, Vol. 2, Section 20, Tremolieres, J., ed., Pergamon Press, New York, NY pp. 465-505, 1970.

Leaf, G. and Zatman, L.J. A study of the conditions under which methanol may exert a toxic hazard in industry. Brit. J. Ind. Med. 9:19-31, 1952.

Ley, C.O. and Gali, F.G. Parkinsonian syndrome after methanol intoxication. Eur. Neurol. 22:405-409, 1983.

Loomis, T.A. Essentials of Toxicology, Lea and Febiger, Philadelphia, PA, 1968.

Makar, A.B., Tephly, T.R., and Mannering, G.J. Methanol metabolism in the monkey. Mol. Pharmacol. 4:471-483, 1968.

Makar, A.B. and Tephly, T.R. Methanol poisoning in the folate-deficient rat. Nature 261:715-716, 1976.

Makar, A.B. and Tephly, T.R. Methanol poisoning VI. Role of folic acid in the production of methanol poisoning in the rat. J. Toxicol. Environ. Health 2:1201-1209, 1977.

Malorny, G. Stoffwechselversuche mit natrium-formiat und ameisensaure beim menschen. (Acute and chronic toxicity of formic acid and formates) Z. Ernaehrungswiss 9:332-339, 1969a. (German)

Malorny, G. Metabolism studies with sodium formate and formic acid in man. Z. Ernaehrungswiss 9:340-348, 1969. (German)

Malorny, G., Rietbrock, N., and Schneider, M. Die oxydation des formaldehyde zu ameisensaure in blut, ein beitrag zum stoffwechsel des formaldehyde. Naunyn-Schmiedebergs Arch. Pathol. Pharmakol. 250:419-436, 1965. (German)

Martin-Amat, G., Tephly T.R., McMartin, K.E., Makar, A.B., Hayreh, M.S., Hayreh, M.S., Baumbach, G., and Cancilla, P. Methyl alcohol poisoning. II. Development of a model for ocular toxicity in methyl alcohol poisoning using the Rhesus monkey. Arch. Ophthalmol. 95:1847-1850, 1977.

Martin-Amat, G., McMartin, K.E., Hayreh, S.S., Hayreh, M.S., and Tephly T.R. Methanol poisoning: Ocular toxicity produced by formate. Toxicol. Appl. Pharmacol. 45:201-208, 1978.

May, J. Odor thresholds of solvents for evaluating solvent odors in air. Staub-Reinhalt Luft 26:385-389, 1966. (German)

McMartin, K.E., Makar, A.B., Martin-Amat G., Palese, M., and Tephly T.R. Methanol poisoning. I. The role of formic acid in the development of metabolic acidosis in the monkey and the reversal by 4-methylpyrazole. Biochem. Med. 13:319-333, 1975.

McMartin, K.E., Martin-Amat, G., Makar, A.B., and Tephly, T.R. Methanol poisoning. V. Role of formate metabolism in the monkey. J. Pharmacol. Exp. Ther. 201:564-572, 1977a.

McMartin, K.E., Martin-Amat, G., Makar, A.B., and Tephly, T.R. Methanol Poisoning: Role of Formate Metabolism in the Monkey. in Alcohol and Aldehyde Metabolizing Systems, Vol. 2, Thurman, R.G., Williamson, J.R., Drott, H., and Chance, B., eds., Academic Press, New York, 1977b, pp. 429-439.

McMartin, K.E., Martin-Amat, G., Noker, P.E., and Tephly, T.R. Lack of role for formaldehyde in methanol poisoning in the monkey. Biochem. Pharmacol. 28:645-649, 1979.

McMartin, K.E., Ambre, J.J., and Tephly, T.R. Methanol poisoning in human subjects: Role of formic acid accumulation in the metabolic acidosis. Am. J. Med. 68:414-418, 1980.

NIOSH, 1976a. National Institute for Occupational Safety and Health. Criteria for a Recommended Standard. Occupational Exposure to Methyl Alcohol. NIOSH-76-148. PB-273 806, 1976.

NIOSH, 1976b. National Institute for Occupational Safety and Health. An Analysis and Critique of Behavioral Toxicology in the USSR. NIOSH-77-160. PB-273 807, 1976.

NIOSH, 1981. National Institute for Occupational Safety and Health (NIOSH). Health Hazard Evaluation Report. HETA 81-177, 178-188 (PB82-194648), 1981.

Nelson, B.K., Brightwell, W.S., Mackenzie, D.R., Khan, A., Burg, J.R., Weigel, W.W., and Goad, P.T. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fund. Appl. Toxicol. 5:727-736, 1985.

Nicho'ls, P. Formate as an inhibitor of cytochrome c oxidase. Biochem. Biophys. Res. Comm. 67:610-616, 1975.

Nicholls, P. The effects of formate on cytochrome aa_3 and on electron transport in the intact respiratory chain. Biochim. Biophys. Acta 430:13-29, 1976.

Noker, P.E., Eells, J.T., and Tephly, T.R. Methanol toxicity: Treatment with folic acid and 5-formyl tetrahydrofolic acid. Alcoholism: Clin. Exp. Res. 4:378-383, 1980.

NEDO. 1986. New Energy Development Oraganization, "Toxicological Research of Methanol as a Fuel for Power Station." September 1986.

NRC. 1983. Committee on the Institutional Means for Assessments of Risks to Public Health. Commission on Life Sciences. National Research Council. Risk Assessment in the Federal Government: Managing the Process. National Academy Press. Washington. DC. 1983.

Opperman. J.A. Aspartame Metabolism in Animals. in Aspartame: Physiology and Biochemistry. Stegink. L.D. and Filer. L.J.. Jr.. eds.. Marcel Dekker. New York. 1984. pp. 141-159.

Osterloh. J.D., Pond. S.M.. Grady. S., and Becker C.E. Serum formate concentrations in methanol intoxication as a criterion for hemodialysis. Ann. Int. Med. 104:200-203. 1986.

Palese. M. and Tephly. T.R. Metabolism of formate in the rat. J. Toxicol. Environ. Health 1:13-24. 1975.

Posner. H.S. Biohazards of methanol in proposed new uses. J. Toxicol. Environ. Health 1:153-171. 1975.

Potts. A.M. The visual toxicity of methanol. VI. The clinical aspects of experimental methanol poisoning treated with base. Am. J. Ophthalmol. 39:86-92. 1955.

Potts. A.M., Praglin. J., Farkas. I., Lowell. M.S., Orbison. L., and Chickering. D. Studies on the visual toxicity of methanol. VIII. Additional observations on methanol poisoning in the primate test object. Am. J. Ophthalmol. 40:76-82. 1955.

Province. W.D., Kritzler. R.A., and Calhoun. F.P. Methyl alcohol poisoning. Report of cases. Bull. U.S. Army Med. Dept. 5:114-116. 1946.

Ranney. R.E.. Oppermann. J.A., Muldoon. E., and McMahon. F.G. The comparative metabolism of aspartame in experimental animals and man. J. Toxicol. Environ. Health 2:441-451. 1976.

Rannug. U. Data from short-term tests on motor vehicle exhausts. Environ. Health Perspect. 47:161-169. 1983.

RD. 1986. Flexible fuel vehicle could help popularize benefits of methanol. Res. Dev., August. 1986.

Reed. T.B. and Lerner. R.M. Methanol: A versatile fuel for immediate use. Science 182:1299-1304. 1973.

Reynolds. W.A., Bauman. A.F., Stegink. L.D., Filer. L.J., Jr., and Naidu. S. Developmental Assessment of Infant Macaques Receiving Dietary Aspartame or Phenylalanine. in: Aspartame: Physiology and Biochemistry. Stegink. L.D. and Filer. L.J., Jr., eds., Marcel Dekker. New York. 1984. pp. 405-423.

Rietbrock. N. Formaldehydroxydation bei der ratte. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. 251:189-201. 1965. (German)

Rietbrock. N. Kinetic und wege des methanolumsatzes. (Kinetics and pathways of methanol metabolism). Naun-Schmied Arch Pharm Exp Path 263:88-105. 1969. (German)

Rowe. V.K. and McCollister. S.B. Alcohols. In Patty's Industrial Hygiene and Toxicology. Third Edition. Vol. 2C. (Clayton. G.D. and Clayton. F.E., eds.) John Wiley & Sons. New York. pp. 4528-4541. 1982.

Roe. O. The metabolism and toxicity of methanol. Pharmacol. Rev. 7:399-412. 1955.

Sayers. R.R.. Yant. W.P., Schrenk. H.H., Chornyak. J., Pearce. S.J., Patty. F.A., and Linn. J.G. Methanol poisoning. I. Exposure of dogs to 450-500 ppm methanol vapor in air. Report of Investigations RI 3617. U.S. Dept. of Interior. Bureau of Mines. pp. 1-10. 1942.

Sayers. R.R.. Yant. W.P., Schrenk. H.H., Chornyak. J., Pearce. S.J., Patty F.A., and Linn. J.G. Methanol poisoning. II. Exposure of dogs for brief periods eight times daily to high concentrations of methanol vapor in air. J. Industr. Hyg. Toxicol. 26:255-259. 1944.

Scherberger. R.F., Happ. G.P., Miller. F.A., and Fassett. D.W. A dynamic apparatus for preparing air-vapor mixtures of known concentrations. Am. Ind. Hyg. Assoc. J. 19:494-498. 1958.

Sedivec. V., Mraz. M., and Flek. J. Biological monitoring of persons exposed to methanol vapours. Int. Arch. Occup. Environ. Health 48:257-271. 1981.

Sejersted. O.M., Jacobsen. D., Ovrebo. S., and Jansen. H. Formate concentrations in plasma from patients poisoned with methanol. Acta Med. Scand. 213:105-110. 1983.

Stegink. L.D. Aspartame metabolism in humans: Acute dosing studies. in Aspartame: Physiology and Biochemistry. Stegink. L.D. and Filer. L.J., Jr., eds., Marcel Dekker. New York. 1984. pp. 509-553.

Stegink. L.D., Brummel. M.C., McMartin. K., Martin-Amat. G., Filer. L.J., Jr., Baker. G.L., and Tephly. T.R. Blood methanol concentrations in normal adult subjects administered abuse doses of aspartame. J. Toxicol. Environ. Health 7:281-290. 1981.

Stegink. L.D., Brummel. M.C., Filer. L.J., Jr., and Baker. G.L. Blood methanol concentrations in one-year-old infants administered graded doses of aspartame. J. Nutr. 113:1600-1606. 1983.

Suomi. S.J. Effects of Aspartame on the Learning Test Performance of Young Stumptail Macaques. in Aspartame: Physiology and Biochemistry. Stegink. L.D. and Filer. L.J., Jr., eds., Marcel Dekker. New York. 1984. pp. 425-445.

Tephly. T.R. Introduction: Factors in Responses to the Environment. Fed. Proc. 36:1627-1628. 1977.

Tephly. T.R., Parks. R.E., and Mannering. G.J. Methanol metabolism in the rat. J. Pharmacol. Exp. Ther. 143:292-300. 1964.

Tephly. T.R. and McMartin. K.E. Methanol Metabolism and Toxicity. in Aspartame: Physiology and Biochemistry. Stegink. L.D. and Filer. L.J., Jr., eds., Marcel Dekker. New York. 1984. pp. 111-140.

Tonning, D.J. Methyl alcohol poisoning. A survey of thirty cases. Nova Scotia Med. Bull. 24:1-8, 1945.

Tyson, H.H. Amblyopia from inhalation of methyl alcohol. Arch Ophthamol. 16:459-471, 1912.

Tyson, H.H. and Schoenberg, M.J. Experimental researches in methyl alcohol inhalation. J. Am. Med. Assoc. 63:915-922, 1914.

Ubaydullayev, R. A study of hygienic properties of methanol as an atmospheric air pollutant, in Levine BS (transl): USSR Literature on Air Pollution and Related Occupational Diseases--A Survey 17:39-45, 1963.

White, L.R., Marthinsen, A.B.L., Richards, R.J., Eik-Nes, K.B., and Nilsen, O.G. Biochemical and cytological studies of rat lung after inhalation of methanol vapor. Toxicol. Lett. 17:1-5, 1983.

Wood, C.A. Death and blindness as a result of poisoning by methyl, or wood, alcohol and its various preparations. Int. Clin. 16:68-78, 1906.

Wood, C.A. Death and blindness from methyl or wood-alcohol poisoning with means of prevention. J. Am. Med. Assoc. 59:1962-1966, 1912.

Wood, C.A. and Buller, F. Poisoning by wood alcohol: Cases of death and blindness from Columbian spirits and other methylated preparations. J. Am. Med. Assoc. 43:972-977, 1904.

Yant, W.P. and Schrenk, H.H. Distribution of methanol in dogs after inhalation and administration by stomach tube and subcutaneously. J. Ind. Hyg. Toxicol. 19:337-345, 1937.

Ziegler, S.L. The ocular menace of wood alcohol poisoning. J. Am. Med. Assoc. 77:1160-1166, 1921.

Zitting, A. and Savolainen, H. Biochemical effects of subacute formic acid vapor exposure. Res. Commun. Chem. Pathol. Pharmacol. 27:157-162, 1980.

A NOTE ON PROCESS

In the spring of 1984, HEI held an initial seminar in Albuquerque, New Mexico to examine the issue of methanol vapor emissions from motor vehicles. The workshop led HEI to commission an overview paper by Dr. Thomas Tephly, an internationally known expert in this area. This paper, which was completed in late 1984, was an essential aid to the Institute's Health Research Committee in defining areas of further inquiry. The staff of the Institute, operating at the direction of the Health Research Committee, then contracted with the Environ Corporation, a health and environmental consulting firm in Washington, D.C., to pull together the published literature on the subject. This report, which was completed in the winter of 1986, formed the basis for another review of the subject at the Health Research Committee's spring 1986 meeting. At that meeting, the Health Research Committee requested the HEI Board of Directors to ask the Administrator of the Environmental Protection Agency (EPA) for his views on the priority of this issue from his perspective. A May, 1986 letter from HEI's chairman did this. Further, at HEI's request, representatives from EPA's Office of Health Research and Office of Mobile Sources attended the Health Research Committee's June 1986 meeting to discuss this issue. Both in person and in a letter from EPA's assistant administrator for research and development, the EPA reaffirmed the high priority of its interest in the development of methanol fuel. Accordingly, the Health Research Committee recommended to the Board that it authorize an analysis that would present HEI's sponsors and the public with a careful appraisal of the current health evidence and its implications, as well as research opportunities that could be implemented by the scientific community, including HEI.

The Health Research Committee wishes to acknowledge and thank a number of contributors to the development of this report, including the Environ Corporation, Dr. Morton Grant, Dr. Dag Jacobsen, Dr. David Leith, Dr. Kenneth McMartin, Dr. Thomas Tephly, Dr. Peter Valberg, and Dr. Myron Wolbarscht. Ms. Jessica Schwartz edited this document. The Committee would like to join the Board in congratulating Dr. Robert Kavet for his outstanding work as primary author of this analysis.

FOR FURTHER INFORMATION CONTACT:

The HEALTH EFFECTS INSTITUTE
215 First Street
Cambridge, MA 02142
(617) 491-2926

HEALTH EFFECTS INSTITUTE
215 First Street • Cambridge, MA 02142
(617) 491-2926

SPECIAL REPORT: May 1987