HEALTH EFFECTS INSTITUTE

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Automotive Methanol Vapors and Human Health:
An Evaluation of Existing Scientific Information
And Issues for Future Research

Report of the Institute's
Health Research Committee

May 1987

The Health Effects Institute (HEI) is a non-profit corporation founded in 1980 to assure that objective, credible, high-quality scientific studies are conducted on the potential human health effects of motor vehicle emissions.

Funded equally by the U.S. Environmental Protection Agency (EPA) and 26 automotive manufacturers or marketers in the United States, HEI is independently governed. Its research projects are selected, conducted, and evaluated according to a careful public process, including a rigorous peer review process, to assure both credibility and high scientific standards.

HEI makes no recommendations on regulatory and social policy. Its goal, as stated by former EPA Administrator William D. Ruckelshaus, is "simply to gain acceptance by all parties of the data that may be necessary for future regulations."

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STATEMENT FROM THE HEI BOARD OF DIRECTORS

The manufacturers of motor vehicles and the Environmental Protection Agency (EPA) have a responsibility under the Clean Air Act to ensure that any new technology affecting mobile source emissions will not pose an "unreasonable risk to the public health." (Section 202 (a) (4)). EPA has been requesting the Health Effects Institute (HEI) since 1983 to undertake a research program to determine what emissions-related health problems, if any, would emerge if methanol were to become more widely used as an automotive fuel.

Methanol-fueled vehicles emit both formaldehyde and methanol vapors. In 1985, HEI began to fund a research program to investigate the potential health effects of aldehydes, including formaldehyde. The HEI Health Research Committee, with our approval, decided to undertake additional analysis before proceeding with research on methanol vapors. This report contains the Committee's assessment. We are publishing it because we believe it will provide considerable guidance to government and industry, as policies relating to methanol fuel are considered.

The report focuses on the potential health effects of exposure of the general public to methanol vapor that might result from an introduction of methanol-powered motor vehicles in the general fleet. The report also evaluates, insofar as present knowledge permits, the likely health implications of such exposure. The report excludes analysis of the effects of accidental spills, ingestion, and worker exposure.

We have examined the report carefully, and we believe it represents a responsible summary of the current state of knowledge about the effects of methanol and the likely impact of exposure to its vapors. The Health Research Committee's analysis of the available evidence indicates that chronic exposure of people to low levels of methanol emissions is not likely to trigger known mechanisms of methanol's toxicity.

There has not been, however, sufficient research to eliminate entirely the possibility that health effects could occur at low levels of chronic exposure to methanol. A study of non-human primates chronically exposed to methanol vapor at moderate to high concentrations was recently completed in Japan. The summary report of that study, although rather sketchy, indicates the possibility of biological effects at exposure levels toward the upper end of the range of levels that have been predicted to arise from vehicular emissions. Accordingly, the specific findings of this study must be obtained, clarified if possible — a difficult and time-consuming task — and the research pressed further, if necessary. This appears particularly important if one goes beyond the foreseeable future and contemplates the immense scale of methanol use that would result if methanol were to become a dominant fuel in the next century.

On balance, we believe that, given the anticipated uses of methanol as a motor vehicle fuel in the foreseeable future, the weight of available scientific evidence indicates that exposure to methanol vapors is not likely to cause adverse health effects. Health concerns regarding methanol vapor should not prevent government and industry from encouraging the development and use of methanol fuels, assuming that such development and use are otherwise in the public interest.

The Health Effects Institute and other research organizations are continuing to investigate the potential health effects from increased formaldehyde emissions that may result from methanol's use. The results of those inquiries will become available over the next several years. We believe that prudent public policy suggests that an additional modest research investment be made by appropriate research institutions, and perhaps by HEI, to reduce uncertainties further in estimating the health risks of low-level exposures to methanol, and to enhance the public's confidence in methanol technology.

Problems at relatively minor usage levels might only become evident as billions of gallons are introduced annually. It seems wise to ensure now that the possibility of adverse health consequences is minimized. It is in this light that any further research is prudent. But our best current assessment is that methanol fuel, under intended conditions of use, does not pose an unreasonable risk to the public health attributable to emission of methanol vapors from the tailpipe of motor vehicles.

In addition to thanking the entire HEI Health Research Committee for its efforts in shaping this document, we would particularly like to thank Dr. Walter Rosenblith, Chairman of the Committee, who directed this effort, Dr. Robert Kavet, who was the primary author of this report when he served as Senior Staff Scientist at HEI, and Dr. Roger McClellan, who gave generously of his time to ensure the quality of this effort.

Archibald Cox, Chairman
William O. Baker
Donald Kennedy
Charles W. Powers

May, 1987

AUTOMOTIVE METHANOL VAPORS AND HUMAN HEALTH:

An Evaluation of Existing Scientific Information And Issues for Future Research

EXECUTIVE SUMMARY

Methanol has the potential to become a major automotive fuel in the United States in the next century. One attractive feature linked to methanol's use is that emissions from methanol-fueled vehicles are expected to result in ambient concentrations of criteria pollutants that are no greater than and, quite likely, lower than those that result from gasoline or diesel emissions. However, the introduction of methanol also may result in increased exposure of the public to methanol and formaldehyde, both currently unregulated. The Environmental Protection Agency (EPA) has identified the importance of technically evaluating these relevant health issues. The Health Effects Institute (HEI) shares the EPA's concern and already has initiated laboratory research to investigate the health effects of aldehydes.

This report, prepared by HEI at the EPA's request, evaluates specifically the health consequences to humans that may result from inhalation of methanol vapors either emitted from methanol-fueled vehicles or during self-service refueling. The report's objectives are (1) to review the nature and mechanisms of methanol's toxicity, (2) to evaluate whether or not methanol's known effects might be expected at the anticipated low levels of intermittent exposure associated with increased use of methanol as a vehicle fuel, and (3) to identify both the areas in which critical knowledge is lacking and the research that could supply the needed information.

Anticipated Exposure Levels of Methanol

Data that estimate the range of potential exposure concentrations of methanol are essential to establish whether or not particular biologic or health effects are likely to occur. The EPA has conducted studies that project concentrations of methanol that will occur in a variety of scenarios. These include (1) three traffic situations - street canyon, roadway tunnel, and expressway; (2) exposures in both public parking and personal garages; (3) and exposures during self-service refueling. The analyses take into account both the driving conditions and the vehicle operating mode, as well as the contribution to emissions of vehicles that are not operating according to certification standards.

The highest exposures are expected in the garage scenarios, particularly the personal garage. Worst-case exposure will probably occur in the personal garage immediately after ignition turn-off when a vehicle produces "hot-soak" evaporative emissions. In most cases, however, personal garage exposures are unlikely to last more than several minutes. The EPA projects that worst-case (i.e., hot-soak) personal garage exposure levels (those from a malfunctioning vehicle in an unventilated garage) may be as high as approximately 240 milligrams per cubic meter (mg/m^3) of methanol, but that, under more realistic conditions (normal ventilation), levels are unlikely to exceed 130 mg/m^3. For the traffic situations evaluated, methanol concentrations are projected to be much lower (less than 6 mg/m^3), even if the fleet is 100% methanol-fueled. One other exposure situation that merits attention is that in which a customer at a self-service filling station will be exposed to roughly 50 mg/m^3 of methanol vapor for 3 to 4 minutes during refueling. The personal garage and self-service refueling scenarios are important not only because they represent relatively high exposure levels, but also because the methanol concentration, in these cases, is independent of the penetration of methanol-fueled vehicles into the fleet. As a point reference, the American Congress of Governmental Industrial Hygienists' (ACGIH) threshold limit value (TLV) for occupational exposure is 260 mg/m^3 (200 ppm); this standard is a time-weighted average for an 8-hour period.

Toxicity of Methanol

Nearly all of the available information on methanol toxicity in humans is related to the consequences of acute, rather than chronic, exposures. Acute methanol toxicity evolves in a fairly well-defined pattern. A toxic dose results from intake of a large quantity of methanol in a short period of time, and initially produces a transient, mild depression of the central nervous system. An asymptomatic latent period follows, and may last from several hours to two days or more. The latent period gives way to the onset of a syndrome that consists of an uncompensated metabolic acidosis with superimposed toxicity to the visual system. The physical symptoms, in severe cases, may progress to coma and death; for those who survive, the visual symptoms may, within days to weeks, reverse or progress to permanent visual impairment. The effects that appear after the latent period are attributable to metabolites of methanol, most prominently, formic acid (which dissociates to formate plus a hydrogen ion), and not to methanol itself.

The minimum lethal dose of methanol (in the absence of medical treatment) ranges between 0.3 and 1.0 grams per kilogram of body weight (g/kg). The maximal dose of methanol expected in the EPA's exposure scenarios, by comparison, is under 1 milligram per kilogram of body weight (0.001 g/kg). However, the clinical literature indicates that susceptibility to methanol's sub-lethal acute effects may vary widely among individuals. Two of the known determinants of susceptibility are (1) co-exposure to ethanol, which greatly slows methanol's entrance into its metabolic pathway, and (2) the level of liver folate, which is crucial to the oxidation of formate, the key toxic metabolite of methanol.

Until the 1950s, a major obstacle to understanding and treating methanol poisoning was the lack of understanding of some of the mechanisms of methanol toxicity. This situation existed because of the lack of appreciation that non-primate species are not suitable models of acute human methanol toxicity. Gilger and Potts, in 1955, demonstrated that, of all common laboratory species tested, only non-human primates experience methanol toxicity, including ocular pathology, which is characteristic of humans. The non-human primate model has been confirmed, and has enabled a systematic exploration of the metabolic bases, kinetics, and mechanisms of methanol's acute toxic syndrome.

Data on humans or non-human primates exposed to low levels of methanol vapors are scarce and not well-developed. The epidemiologic literature provides weak suggestive evidence that prolonged occupational exposure to methanol vapors at levels above the TLV (260 mg/m³) may produce symptoms such as headache and blurred vision. However, the conclusions are based on symptom reporting, a less preferable source of data than clinical examination, and assessments of exposures are generally inadequate.

In human clinical experiments, two separate Russian studies reported effects of low-level, short-duration methanol exposures (less than 10 mg/m³, approximately 5-minute exposures) on neurobehavioral endpoints, specifically, dark adaptation and EEG-conditioned thresholds. These reports, however, fail to provide descriptions of critical methodological and analytical procedures, as well as complete descriptions of study subjects, and provide only limited data that describes the results. Upon close examination, the results from the two studies are not consistent, and they are not entirely plausible.

In acute and chronic animal experiments published to date in the peer-reviewed scientific literature, there are no indications that adverse health effects are expected at the potential methanol exposure levels discussed earlier. In Japan, the Institute for Applied Energy, with sponsorship of the New Energy Development Organization (NEDO), conducted an extensive research program in which rodents and non-human primates were exposed to methanol vapors either briefly or for extended periods of time. Although the report issued by NEDO indicates potential effects to the central nervous system of non-human primates exposed to 13 mg/m³ for extended periods of time, the details available in that document are insufficient to permit critical evaluation. Further evaluation of these studies will be necessary.

Finally, in people, both methanol and its toxic metabolite, formate, are present at background levels that result from normal dietary intake and natural metabolic processes. A major contributor to the body burden of methanol in many people is the artificial sweetener, aspartame, now found in many foods. Following ingestion, 10% of the aspartame molecule enters the circulation as methanol.

Metabolism of Methanol and Mechanisms of Toxicity

Methanol distributes readily and uniformly to all organs and tissues in direct relation to their water content. For short-term inhalation exposures, an upper-bound estimate of initial body burden assumes total absorption of the inhaled vapor. A 70 kg person breathing at a ventilation rate of 20 m³/day (twice resting), who is exposed to 200 mg/m³ methanol vapor for 15 minutes (as in a worst-case hot-soak garage scenario), accumulates a methanol body burden of 0.0006 g/kg — at least 500 times lower than doses of acute clinical significance.

Following its uptake and distribution, methanol clears from the human body with half-times of a day or more for high doses (greater than 1 g/kg), and about three hours for the low doses of relevance to this report's objectives (less than 0.1 g/kg). Methanol is either excreted unchanged, mainly in urine and exhaled breath, or enters a metabolic pathway (in the liver) whose ultimate products are carbon dioxide (which is exhaled harmlessly) and water. For the body burdens of methanol that follow a worst-case exposure, metabolism is the dominant pathway, accounting for over 90% of methanol's clearance. This is a key point because methanol's toxic properties are linked to intermediate metabolites, not to the alcohol itself.

In all mammalian species studied, the sequence of metabolic intermediates leading from methanol to its end products is the same:
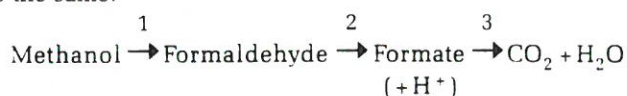
$$\text{Methanol} \xrightarrow{1} \text{Formaldehyde} \xrightarrow{2} \text{Formate} \xrightarrow{3} CO_2 + H_2O \ (+H^+)$$

The toxic properties of methanol, and the basis of species susceptibility, are rooted in the factors that govern the relative rates of formic acid generation (steps 1 and 2) and formate oxidation (step 3). In short, the toxic syndrome sets in if formate generation continues at a rate that exceeds its rate of metabolism to carbon dioxide (CO_2). This imbalance, if protracted, leads to an accumulation of formate coupled, eventually, to an uncompensated metabolic acidosis. The acidosis, if untreated, can prove lethal; formate, even at physiologic pH, is associated with ocular toxicity. In both rats, which are methanol-resistant, and in non-human primates, which are susceptible, the folate pathway in the liver mediates formate metabolism to carbon dioxide. The efficiency of this process is linked to the availability of tetrahydrofolate (THF), the molecule that initially complexes with formate. Non-primate species dispose of formate efficiently at any dose and, thus, escape toxicity, whereas, at sufficiently high doses, humans and non-human primates accumulate toxic metabolites and, thus, are at risk to adverse consequences. As an aside, formaldehyde is not believed to play a role in methanol toxicity.

The mechanisms responsible for injury to the visual system in acute methanol poisonings are not yet understood, but several investigators have postulated that formate, at sufficiently high blood levels, may inhibit cellular respiration in the proximal portion of the optic nerve, leading to a compression type of injury to the nerve's axons that ultimately affects vision. An acidotic state may accelerate such an injury.

Although formate possesses toxic potential, the levels it will achieve in people following worst-case environmental expo-

sures to methanol will not come close to challenging the metabolic capacity of the folate pathway. The small increases of formate levels that have been observed in the blood and urine of adult humans following either occupational exposure to methanol vapors or experimental administration of aspartame reflect normally operating metabolic pathways. The blood levels of formate that follow worst-case (i.e., hot-soak, personal garage) environmental exposure to methanol vapor will, in all likelihood, not be discriminated from the background level of blood formate.

Evaluation and Recommendations

• Discussion

The implementation of methanol as a vehicle fuel is likely to increase the exposure of the general public to methanol vapors. EPA analyses predict that the highest exposure levels will occur in personal garages during engine hot-soak, at the self-service pump during refueling, and, with increasing penetration of methanol technology into the fleet, in public parking garages. By comparison, exposure concentrations in traffic situations, even with 100% penetration of methanol-fueled vehicles, will be very low.

The health effects of methanol are best recognized and studied for cases in which subjects have orally ingested large single doses. The clinical literature documents many case histories of methanol poisoning; its course, which consists of metabolic acidosis and visual disturbance that follow a symptomless latent period, is well characterized. Methanol's toxicity, in these cases, is attributable to its metabolite, formate. Methanol as an unmetabolized substance is not considered toxic unless it is taken in narcotic doses. The discovery, in the 1950s, of the non-human primate as a model of acute human toxicity was perhaps the single most important event to lead to our current understanding of methanol's acute toxicity.

The characteristics of methanol's chronic effects, on the other hand, are not well known. The literature from studies of non-human primates is of little value in evaluating the dose- and time-effect characteristics for protracted exposures of people. The limited evidence from epidemiologic studies and case reports suggests that chronic effects, if they appear, are similar to those described for acute toxicity (e.g., headache, blurred vision), but are less severe. Thus, acute and chronic effects may share common pathways of action. In the small number of instances that report chronic effects attributable to methanol, exposure levels exceed the ACGIH TLV of 260 mg/m^3.

In the worst-case exposure scenario (hot-soak, personal garage), the inhaled body burden of methanol (0.0006 g/kg)

will be approximately equivalent to the pre-existing background levels of methanol (0.0005 g/kg) for a brief period of time following exposure. For self-service refueling, the contribution will be roughly 10 times less. The average daily intake of methanol from aspartame in the diet (approximately 0.0003 to 0.0015 g/kg) is on the same order of magnitude as uptake from a single worst-case exposure in the hot-soak garage. Even more importantly, however, worst-case methanol exposures will not lead to blood formate levels that challenge the folate pathway's capacity to oxidize formate. Furthermore, the increase expected in blood formate following worst-case exposure will be negligible in comparison to the background levels of blood formate.

• Conclusion

Based on the foregoing evidence, if methanol produces health effects in normal subjects at or near the exposure levels of concern, these effects would not likely be attributable to the generation of formate. However, the effects of low-level formate accumulation in potentially susceptible subjects has not been examined.

A firm conclusion about the potential health effects from chronic exposures cannot be drawn yet. To date, no human epidemiologic studies have reported effects that could be linked to chronic methanol exposures below the TLV of 260 mg/m^3. However, careful investigations of people exposed chronically to levels below the TLV are not available, and would, no doubt, prove very useful if the levels of exposure were rigorously quantitated.

An analysis of the available peer-reviewed literature produces no evidence upon which to base a conclusion that exposure to methanol vapors will result in adverse health effects. This conclusion applies only to exposures that will occur as a result of methanol's normal use as a vehicular fuel, and not to exposure that may occur either from ingesting methanol fuels or from spillage.

Although adverse effects have not been indicated in this analysis, further research targeted to answer specific questions would help in further reducing the uncertainties of estimating the health effects of protracted or repeated low-level exposures, and would serve to reinforce the certainty of conclusions about the public's health. Such research should attempt to elucidate the potential consequences of protracted or repeated low-level exposure, using human epidemiologic approaches and animal experimentation. In the latter, further work could be conducted that would more completely describe the dose- and time-effect relationships between formate concentrations in the body and effects to the visual system. Achieving these objectives would lead to a better understanding of metabolic processes in suspected target tissue.

AUTOMOTIVE METHANOL VAPORS AND HUMAN HEALTH:

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I. INTRODUCTION

OBJECTIVES OF THE REPORT

Intense interest surrounds the potential use of methanol as a principal automotive fuel for the United States in the next century (Anderson. 1984; Gray and Alson, 1985; ARB, 1986). Several experimental fleets of methanol-fueled vehicles are already in operation, and others are planned (ARB, 1986; RD, 1986; CEN, 1986). The Clean Air Act requires that it be established that implementing such a technology on a mass basis will not degrade environmental quality or compromise the public health. Although substituting methanol for currently used fuels is expected to reduce ambient concentrations of criteria pollutants such as particulate matter, oxides of nitrogen, and ozone (Gold, 1985; ARB, 1986), its use may increase airborne concentrations of two gaseous pollutants of potential concern: formaldehyde and methanol (Harvey et al. 1984). Methanol emissions arise from their release as uncombusted material in the exhaust and also from their evaporation during refueling, and following ignition turn-off; evaporative emissions also occur during the daily heating of the fuel tank. Formaldehyde is a by-product resulting from the incomplete combustion of methanol.

This report focuses on the health issues associated with exposure of the public to methanol vapors emitted from methanol-fueled vehicles. Its objectives are (1) to review the nature and mechanisms of methanol toxicity, (2) to evaluate whether or not methanol's known effects might be expected at anticipated levels of exposure, and (3) to identify both the areas in which critical knowledge is lacking, and the research that could supply the needed information. Although issues such as spillage, oral ingestion of methanol's liquid form, and occupational exposures to methanol vapors are not targeted specifically, the analyses presented may, nonetheless, be useful to these situations as well. [The Health Effects Institute is also keenly interested in furthering knowledge of the potential effects of formaldehyde in the context of exposure to emissions from both conventional and methanol-fueled vehicles. In pursuit of that end, HEI issued Requests for Applications (RFA) to the scientific community in 1985 to study formaldehyde's health effects. The research based on that RFA recently has recently been initiated.]

ORGANIZATION OF THE REPORT

A comprehensive assessment of the potential health impacts of exposure to methanol vapor and a statement of recommendations for further research require integration of information from a variety of sources. Therefore, the next three sections of this report present topics and issues that constitute the building blocks of an environmental health risk assessment of methanol. The final section organizes and synthesizes the technical material in an analytical framework, and provides conclusions and recommendations. A brief description of each section follows:

Section II - *Methanol's Fuel Properties and Anticipated Ambient Levels:* This section reviews the characteristics of methanol that recommend it as a vehicle fuel, and summarizes studies conducted by the EPA that project the ambient concentrations of methanol that will result from its use. These projections cover various exposure scenarios (e.g., expressway, personal garage) and are critical to help assess whether or not people are likely to experience health effects under anticipated exposure conditions.

Section III - *Toxicity of Methanol:* This section discusses the signs and symptoms of methanol toxicity, a subject of numerous publications in the toxicologic and clinical literature since the turn of the century. This literature is almost exclusively concerned with the acute effects of methanol poisoning following brief intakes of large quantities of methanol, and supplies very little information about chronic, low-level exposure. The section highlights the differences between primate and non-primate species in terms of their sensitivity to acute methanol toxicity. Such distinctions are important for subsequent discussions of the metabolism of methanol and its toxic mechanisms in humans. Appendices are provided (a) to review the literature that deals with long-term or repeated human exposures to methanol, (b) to describe in detail two Russian human clinical studies, and (c) to discuss the limited literature on chronic exposures of animals to methanol.

Section IV - *Metabolism of Methanol and Its Toxic Mechanisms:* The uptake, distribution, and subsequent fate of methanol and its metabolites are described. The discussion includes the biochemical basis for the interspecies differences in susceptibility presented in Section III. This material provides the underpinnings for dose-response relationships whose understanding is vital to the overall purposes of this report.

Section V - *Evaluation and Recommendations:* Here, the information from the preceding four sections is synthesized to project the extent to which known toxic processes may occur under predicted ambient exposure conditions. The analysis considers inhalation as the principal route of exposure, as well as the metabolic and excretory pathways described in Section IV. Finally, the section identifies areas in which the health database might be improved, and advances recommendations for research opportunities that can help in further reducing uncertainties in estimating the health effects of protracted or repeated low-level exposures to methanol.

II. METHANOL'S FUEL PROPERTIES AND ANTICIPATED AMBIENT CONCENTRATIONS

METHANOL AS A FUEL

Methanol (CH_3OH), also called methyl alcohol, is the simplest of all the alcohols. In its pure form, it is a clear, colorless liquid at room temperature, and has the physical and chemical characteristics listed in Table 1. Additional characteristics of methanol relate to its use as a motor vehicle fuel. Pure methanol has an octane rating of between 106 and 115, and an energy content of about 16 kjoule/cm³; in-use gasoline fuels have octane ratings ranging from 87 to 94, and an energy content roughly twice that of methanol (Reed and Lerner, 1973; ARB, 1986).

Table 1. Physical and Chemical Properties of Methanols

Physical state	Colorless, volatile liquid
Molecular formula	CH_3OH
Molecular weight	32.04
Boiling point	64.7°C
Melting point	− 97.8°C
Specific gravity	0.7915 (@ 4°C)
Vapor pressure	160 mm Hg (@30°C)
Refractive index	1.3292 (@ 20°C)
Flammability	
Flash point	12°C
Explosive limits	6.0 to 36.5% volume in air
Ignition temperature	470°C
Percent in saturated air	21.05 (@ 30°C)
Density of saturated air (air = 1)	1.02
Solubility	Miscible with water, alcohols, ketones, esters, halogenated hydrocarbons, and benzene
1 mg/ml < >	764 ppm at 25°C, 760 mm Hg
1ppm < >	·1.31 mg/m³ at 25°C, 760 mm Hg

Source: Rowe and McCollister, 1982

From its discovery in the early 19th century to the mid-1920s, methanol was made exclusively from the destructive distillation of wood, which led to its well-known name, wood alcohol, a term that persists to the present. Methanol had a variety of personal, commercial, and industrial uses as a solvent, medicinal agent, and as a source of energy. In the last century, for example, methanol was used in France for lighting, heating, and cooking. Facing gasoline shortages in the 1930s, several European countries equipped civilian and military vehicles with wood-burning units that provided power in the form of alcohol vapors (Posner, 1975). For the past six decades, however, most methanol has been made by reacting carbon monoxide (CO) with hydrogen (H_2) at elevated temperature and pressure, and methanol continues to be used in a wide variety of commercial and industrial applications (e.g., manufacture of formaldehyde, anti-freeze ingredient, denaturant) (Reed and Lerner, 1973; Posner, 1975).

The economic, policy, and social factors connected with methanol's future in the automotive fuel marketplace fall outside of the scope of this report. Suffice to say that methanol combines several important attributes that highlight its potential for mass use, which, in turn, justifies an evaluation of its possible impacts on the public health. Briefly, these attributes pertain to: *Availability* - Methanol's precursors (CO and H_2) are available from a variety of carbonaceous sources, particularly coal, which is in abundant reserve in the United States; *Distribution* - The systems already in place for storage and transport of petroleum products also are able to handle alcohol fuels; *Fleet Adaptability* - Currently used gasoline and diesel engines, with varying amounts of modification, can be converted into efficient methanol-burning engines; also, in-use gasoline engines are adaptable to gasoline-methanol blends; *Air Quality* - Emissions from methanol-fueled vehicles are expected to result in ambient concentrations of criteria pollutants no greater than, and very likely, lower than those that result from gasoline or diesel emissions.

EXPOSURE TO METHANOL VAPOR FROM VEHICLES

Despite projected improvements in air quality relative to regulated pollutants, the introduction of methanol technology may result in an increased exposure of the public to methanol and formaldehyde (Harvey et al, 1984), both of which are currently unregulated as mobile source emissions. The Environmental Protection Agency has correspondingly expressed concern for health effects that may be associated with exposure to each of these substances (Carey, 1983; Harvey, 1983). As mentioned earlier, the Health Effects Institute shares this concern, and already has initiated research on the health effects of formaldehyde. This report, however, evaluates the health consequences of exposure to methanol only.

The critical value of exposure assessments to the characterization of environmental impacts on public health is fully recognized (NRC, 1983). Fortunately, a number of studies conducted by the EPA already have provided data on the ambient concentrations of methanol vapor expected under a wide range of vehicle and traffic conditions (Harvey et al, 1984; Gold, 1985). These data, though not yielding detailed time and activity exposure analyses, have identified situations in which maximal, and perhaps toxicologically relevant, exposures are likely to occur.

The basic approach of these studies is to characterize light- and heavy-duty fleet emissions, and, with air quality modeling programs, use these data to compute expected ambient concentrations of methanol. In the initial steps, exhaust and evaporative emissions from individual vehicles are characterized under various conditions related to both driving cycle and the maintenance level of the vehicle. These data may be derived directly from laboratory testing and measurement of

emissions from sample vehicles. Alternatively. emission values may be based on design targets or pre-set certification standards with offset (i.e.. correction) factors applied to project actual in-use performance. Offset factors may reflect expected changes in emissions associated with tampering with emission control devices. or malfunctions resulting from either lack of maintenance or from random causes. (See Appendix I for a further description of offsets.)

The next objective is to translate vehicle-specific emissions into ambient concentrations of methanol vapor. For one important scenario. the personal garage. the emission characteristics of solitary vehicles (not fleets) and garage size and ventilation rates are the key determinants of methanol concentrations.

For traffic and parking garage situations. characterizations of entire fleets are essential to project air quality data. The EPA has developed MOBILE3. a database program that helps meet this need. MOBILE3 continually updates detailed profiles of the composition and emissions of in-use gasoline and diesel fleets. and allows for projecting fleet characteristics into the future. The data in the program include miles traveled per vehicle type and model year. For the calculation of total fleet emissions. MOBILE3 factors in variables. such as vehicle deterioration. that may affect the final emissions inventories. The data generated on methanol vapor concentrations. described below and in Tables 3 through 5. are extracted from the air quality models developed for gasoline and diesel fleets. (Appendix I further describes the exposure scenarios and the data that appear in Tables 3 through 5.)

The EPA studies have modeled methanol exposure levels that may occur in specific situations representative of the full range of vehicle use and traffic flow. Table 2 lists the exposure scenarios for light-duty vehicles together with the driving cycle and type of emissions (exhaust and/or evaporative) that best characterizes vehicle performance within each scenario. The 'typical' and 'severe' classifications for street canyon, roadway tunnel, and expressway in the table refer to traffic density and road design, as well as to the driving speed and test cycle listed. For example, the typical street canyon scenario that is modeled calls for a sidewalk exposure next to a four-lane street with a traffic load of 800 vehicles per hour; in the severe case, the street is six lanes with 2,400 vehicles per hour.

Although not shown in Table 2. typical and severe emissions also apply to the personal and parking garage scenarios. For personal garages, vehicle warm-up time influences the extent of potential exposure during the idle cycle; for example, in the model, a typical warm-up interval for moderate weather conditions is 30 seconds, and is 5 minutes during severe winter conditions (for both, the garage door is considered to be open). During personal garage hot-soak (evaporation from a hot engine after it has been turned off), the relative severity of exposure is a function of a garage's size and air exchange rate. For parking garages, inflow and outflow rates of traffic, as well as facility size and ventilation, all determine idle and hot-soak concentrations.

Table 2. Scenarios and Appropriate Test Cycles for Evaluating Emissions from the Use of Methanol as a Vehicle Fuel

Scenario	Scenario Average Speed	Suggested Test Cycle	Test Average Speed
Street Canyon			
Typical	5	NYCC[a] (+ SHED)[b]	7.1
Severe	20	FTP[c] (+ SHED)	19.5
Tunnel			
Typical	0-40	FTP (hot)[d]	19.5
Severe	25	FTP (hot)	19.5
Expressway			
Typical	55	HFET[e]	48.2
Severe	50	HFET	48.2
Off Road			
Parking and Personal Garages (idle)	0	Cold Idle	0
Parking and Personal Garages (hot-soak)	0	Hot-Soak (SHED)	0

[a] NYCC: New York City Cycle. a low speed driving cycle (exhaust emissions)
[b] SHED: Sealed Housing for Evaporative Determination. the standard evaporative emissions test
[c] FTP: Federal Test Procedure. a cold start driving cycle simulating typical urban driving (exhaust emissions)
[d] FTP(hot) refers to a hot start FTP (i.e.. vehicle already fully warmed up) (exhaust and evaporative emissions)
[e] HFET: Highway Fuel Economy Test. a driving cycle simulating rural highway driving (exhaust emissions)

Source: Harvey et al. 1984

Tables 3 through 5 display the methanol concentrations expected under the conditions described above. Although the discussion above focused on light-duty vehicles. the values in Table 3 (roadway traffic scenarios) represent combined light- and heavy-duty fleets: Tables 4 and 5, which describe personal and parking garage scenarios, represent exposures from only light-duty vehicles. The tables show that maximal exposures are expected in the garage scenarios, particularly in the personal garage. In a public parking facility, exposure levels may reach as high as 60 mg/m^3 during hot-soak, assuming all of the vehicles in the garage are fueled with methanol. In a normally ventilated personal garage, methanol concentrations produced by hot-soak emissions from a malfunctioning vehicle (i.e., one with a disabled canister) probably will not exceed 150 mg/m^3. For the hypothetical case of hot-soak emissions from a malfunctioning vehicle in an unventilated garage, the methanol concentration may reach as high as 240 mg/m^3, a level that may be considered at the outer limits of exposure. In most cases, however. personal garage exposures will be brief, lasting only for the period that the operator occupies the garage during idle or hot-soak, perhaps

Table 3. Estimated In-use Ambient Methanol Concentrations for Traffic Scenarios — mg/m^3 (100% Fleet Penetration)

Scenario	Likely Certification Level (g/mile)	All Vehicles Meet Standard[1]	25% of Vehicles Malfunction[2]
STREET CANYON			
Typical	0.54	0.04	0.08
Severe		0.25	0.54
ROADWAY TUNNEL			
Typical	0.54	1.0	2.1
Severe		2.5	5.4
EXPRESSWAY			
Typical		0.005	0.19
Severe	0.023	0.019	0.77
Off-road		0.004	0.161

[1] - fleetwide offset = 1.0
[2] - fleetwide offset = 2.2, street canyon
                    2.2, roadway tunnel
                   40.8, expressway

Data adapted from: Gold, 1985 and Harvey et al. 1984

NOTE: For a further explanation of the data, a description of the scenarios, and assignment of offset factors, see Appendix I.

Table 4. Estimated Peak In-use Ambient Methanol Concentrations for Personal Garage — mg/m^3 (Solitary Vehicle)

Mode	Likely Certification Level	Vehicle Meets Standard[1]	Vehicle Malfunctions[2]
IDLE (TRIP START)			
Typical	0.002-0.043 g/min	0.02-0.34	0.54-12
Severe		0.13-2.9	4.6-98
HOT-SOAK (TRIP END)			
Typical	1.1-3.0 g/hr	10-27	48-130
Severe		18-49	86-240

[1] - vehicle offset = 1.0
[2] - idle offset = 34 (non catalyst configuration)
    hot-soak offset = 4.8 (canister disabled)

Data adapted from: Gold, 1985 and Harvey et al. 1984

NOTE: Exposure in the personal garage scenario is brief, probably not lasting more than 15 minutes. For a further explanation of the data, a description of the scenarios, and assignment of offset factors, see Appendix I.

a matter of minutes. For traffic situations (Table 3), methanol concentrations are generally much lower (less than 6 mg/m^3) than in the garages, with maxima predicted for roadway tunnels.

Table 5. Estimated In-use Ambient Methanol Concentrations for Parking Garage — mg/m^3 (100% Fleet Penetration)

Mode	Likely Certification Level	All Vehicles Meet Standard[1]	Vehicle Malfunction[2]
IDLE (TRIP START)[3]			
Typical	0.002-0.043 g/min	0.17	1.6
Severe		2.4	23
HOT-SOAK (TRIP END)			
Typical	1.1-3.0 g/hr	0.30-0.81	0.49-1.4
Severe		13-35	21-58

[1] - fleetwide vehicle offset = 1.0
[2] - fleetwide offset for idle = 9.5 (based on 25% malfunction rate)
    fleetwide offset for hot-soak = 1.7 (based on 10% malfunction rate)
[3] - Idle data based on upper certification level

Data adapted from: Gold, 1985 and Harvey et al. 1984

NOTE: Exposure in the parking garage scenario is brief, probably not lasting more than 15 minutes. For a further explanation of the data, a description of the scenarios, and assignment of offset factors, see Appendix I.

All scenarios except the personal garage assume 100% penetration of methanol-fueled vehicles into the fleet. Thus, all methanol values in Tables 3 and 5 scale linearly with penetration fraction. Despite the brief exposure interval, the personal garage scenario is important not only because it represents the highest exposure level, but also because the methanol concentration is independent of penetration. Although the fleet values (Tables 3 and 5) may not be achieved for decades, if ever, those for personal garages (Table 4) are projected for the individuals driving methanol-fueled cars and using personal garages at any level of penetration. Therefore, the potential exposures in personal garages may be of more immediate concern.

One final exposure situation that merits attention is service-station refueling. According to EPA estimates, a typical methanol fill-up will result in about three to four minutes of exposure to between 33 and 50 mg/m^3 of methanol (Harvey et al. 1984). For self-service customers, such exposures may occur once or twice weekly, but for station attendants exposure will be much more frequent.

The exposure data described above in Tables 3 through 5, and in Appendix I, are summarized in Figure 1. For reference, Figure 1 also indicates that the American Congress of Governmental Industrial Hygienists' (ACGIH) threshold limit value (TLV) for exposure to methanol averaged over an 8-hour work day is 260 mg/m^3 (ACGIH, 1985). This value has been designated for working populations, and not for the general public, for whom the philosophy of standard-setting differs. It is presented only as a point of reference for the ambient exposure data in Figure 1.

METHANOL CONCENTRATION (mg/m³)

Chart axis values: 1,000 — 100 — 10 — 1.0 — 0.1

ACGIH TLV

Data point labels by scenario:

- Street Canyon: SEVERE, 25% MALF, TYPICAL
- Roadway Tunnel: SEVERE, 25% MALF, TYPICAL
- Expressway: SEVERE, 25% MALF, TYPICAL
- Personal Garage (IDLE): SEVERE (~100), MALF, TYPICAL, CERT; (HOT SOAK): SEVERE, MALF, TYPICAL, SEVERE, CERT, TYPICAL
- Parking Garage (IDLE): 25% MALF, SEVERE, TYPICAL; (HOT SOAK): 10% MALF, TYPICAL
- Self-Service Refueling: SEVERE, TYPICAL

Bottom labels: IDLE — HOT SOAK — IDLE — HOT SOAK

SCENARIO

STREET CANYON | ROADWAY TUNNEL | EXPRESS WAY | PERSONAL GARAGE | PARKING GARAGE | SELF-SERVICE REFUELING
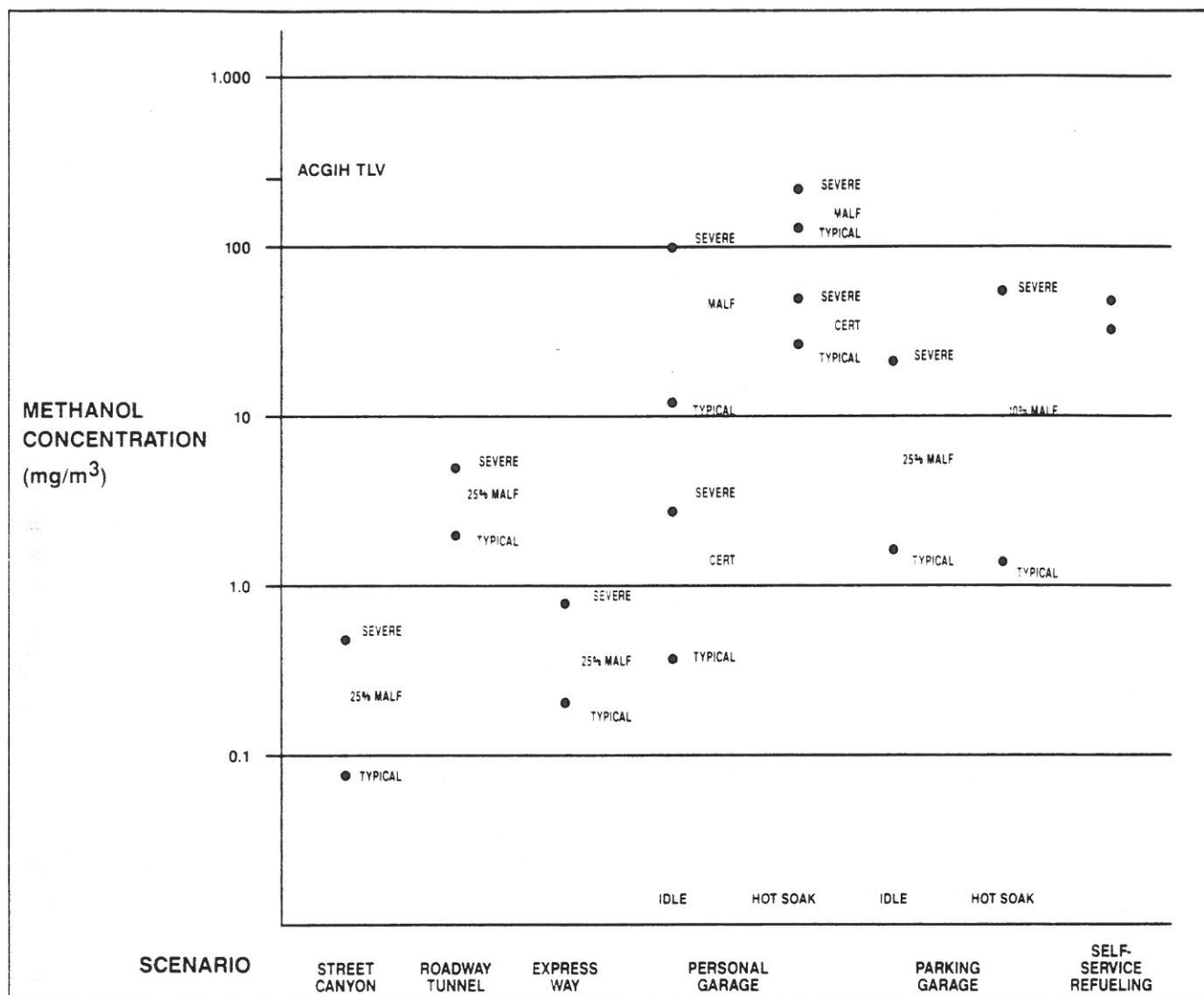
Figure 1: A summary of the data on methanol exposure levels shown in Tables 3 through 5. Severe (S) and Typical (T) relate to conditions described in Section II and Appendix I. For street canyon, roadway tunnel, expressway and parking garage (idle), the data are based on a 25% malfunction (Malf) rate in the fleet. For parking garage (hot soak), the data are based on a 10% malfunction rate. Because a personal garage scenario involves only a single vehicle, data are shown for a malfunctioning vehicle and for one operating according to likely certification standards (Cert). In this case, the above designations do not apply to the refueling scenario.

III. TOXICITY OF METHANOL

BACKGROUND

In the 1890s, the use of wood alcohol increased significantly, and its acute toxicity was fully realized shortly thereafter. Previously, impurities retained in its distillation process rendered wood alcohol a vile-tasting and foul-smelling substance. Human exposures to wood alcohol and its vapors consequently were limited, and reports of intoxication, therefore, were very rare. The introduction of an inexpensive deodorization process during the 1890s increased greatly the market for wood alcohol as a commercial product and as a solvent for use in the workplace. It was sold in stores as a pure substance under various commercial names such as Columbian Spirits, Eagle Spirits, and Lion d'Or, and was included as an ingredient in many other consumer products such as witch hazel, Jamaica ginger, vanilla extract, and perfumes (Jelliffe, 1905; Wood, 1906). Perhaps the most notorious use of wood alcohol was, and continues to be, as an adulterant in alcoholic beverages, a practice that has led to large scale episodes of poisonings since the turn of the century (Chew et al, 1946; Province et al, 1946; Bennett et al, 1953; Kane et al, 1968; Dethlefs and Naraqi, 1978).

In parallel with its spread in the consumer market place, wood alcohol became a widely used substance in the workplace. Tyson and Schoenberg (1914) reviewed data from the 1904 U.S. census, and tallied nearly two million workers in occupations in which wood alcohol was used. Those most heavily represented included: painters, glaziers, and varnishers (278,000); launderers (386,000); boot and shoemakers (200,000); and printers and lithographers (155,000).

The dramatic increase in wood alcohol's distribution and use, coupled with an almost universal ignorance concerning its toxic potential, led quickly to an accumulation of case reports describing wood alcohol poisonings. In 1904, Wood and Buller published an oft-quoted series of 235 case studies that characterize many of the key presenting features of acute methanol poisoning (Wood and Buller, 1904; Buller and Wood, 1904). Briefly, about a day after exposure, victims are stricken with visual disturbances and an array of incapacitating physical symptoms that may lead to coma and death. Wood and Buller and others in the medical community sounded alerts concerning wood alcohol and lobbied in the medical literature for social and legislative actions to control access to wood alcohol and its use (Wood, 1912; Tyson and Schoenberg, 1914; Ziegler, 1921). The vast majority of poisonings in Wood and Buller's reports, and in the many wood alcohol methanol incidents recorded since, have occurred from drinking adulterated beverages or wood alcohol products. In the largest single episode, Bennett et al (1953) describe a case that occurred in Atlanta in 1951 when, within a five day period, 323 people ingested bootleg whisky contaminated with methanol; 41 of these poisonings were fatal.

Although oral ingestion dominates historically as the most frequent route of poisoning, the literature also substantiates that percutaneous absorption of methanol liquids or inhalation of its vapors are as effective as the oral route in producing methanol's acute toxic syndrome (Wood and Buller, 1904; Buller and Wood, 1904; Gimenez et al. 1968). Tyson and Schoenberg (1914) counted about 100 cases reported up to 1912 of amblyopia (impairment of vision) and death from inhalation of wood alcohol vapors. Referring to the case literature on wood alcohol inhalation Ziegler (1921) wrote:

"The majority of these cases occur from occupational exposure to the fumes. The painter uses it as a cleansing fluid or as a cheap diluent to cut his shellac in order to varnish the interior of large beer vats, closets or closed room. Two of Tyson's (1912) patients finally succumbed to the slow poisoning. The hatter mixes it with shellac to stiffen the nap or straw blanks. The dyer of feathers uses it to dilute the colors; the maker of shoe polish adds it to the paste; the brass finisher uses it in the lacquer, and the maker of rubber tires mixes the mass with it. If ventilation is very free, the danger will be lessened; but open air exercise at frequent intervals should be required for every such employee."

ACUTE TOXICITY: DESCRIPTION

Nearly all of the available information about methanol toxicity in humans concerns the consequences of acute exposures.[1] This information is based on clinical case studies recorded since the turn of the century and, more recently, on laboratory experiments that employ valid animal models of human toxicity. The few reports in the human case literature concerning repeated or prolonged exposures suggest that chronic and acute effects may share similar qualities. Therefore, a description of methanol's acute toxic properties is appropriate to this report's objectives.

Acute methanol toxicity in humans evolves in a fairly well-defined pattern. A toxic exposure results initially in a transient, mild depression of the central nervous system (CNS). An asymptomatic latent period follows, and may last from several hours to two days or more, although 12 to 24 hours is most common (Bennett, 1953; Roe, 1955). The latent period gives way to the onset of a syndrome that consists of an uncompensated metabolic acidosis with superimposed toxicity to the visual system. Physical symptoms typically may include headache, dizziness, nausea, and vomiting; these may be followed by severe abdominal pain and difficult, periodic breathing (Kussmaul breathing), which may progress to coma and death, usually from respiratory failure (Tephly and McMartin, 1984; Jacobsen and McMartin, 1986).

In parallel with the onset of these symptoms, subjects experience visual disturbances that include blurred or indistinct vision and altered visual fields (often depression of the central field), and, in severe cases, total blindness (Chew et al. 1946; Bennett et al, 1953). Impairment of the pupillary response to light usually accompanies the visual symptoms, and the extent of impairment is predictive of survival. Subjects with unresponsive, dilated pupils often succumb to the toxic syndrome, and those who survive suffer appreciable and, in many cases, permanent loss of vision.

Ophthalmoscopic examinations of methanol-poisoned victims show that hyperemia (i.e., a local increase in blood flow) of the optic disc is the earliest change that occurs in the retina; hyperemia accompanies the initial visual symptoms (Benton and Calhoun, 1952; Dethlefs and Naraqi, 1978). Within a day, a white striated edema (an accumulation of an excessive amount of watery fluid) appears that projects into the surrounding retina from the optic disc, whose margin simultaneously acquires a blurred appearance; the papilla itself is not edematous. (The papilla, also called the nerve head, is the area where the nerve fibers of the retina converge to form the optic nerve.) The optic disc hyperemia usually subsides within a week, but edema in the region of the optic disc may persist for up to two months. The edema follows the course of major blood vessels and appears to be located mainly in the nerve fiber layer of the retina.

In the Atlanta epidemic, these ophthalmoscopically visible changes were observed in 87% of patients with acute visual symptoms, and in all patients who developed permanent visual deficit. Furthermore, the severity of retinal edema was predictive of restoration of vision; mild edema resulted frequently in full recovery, and severe edema led invariably to permanent effects (Benton and Calhoun, 1952). Pallor of the optic disc is an end-stage sign of irreversible effects to the visual system, and may appear one to two months after an acute methanol dosage (Wood and Buller, 1904; Buller and Wood, 1904; Bennett et al, 1953) [or possibly following chronic

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[1]In this report, the terms acute and chronic refer to the time-course of exposure, rather than to the time-course of the appearance of effects. The reader should bear this distinction in mind as methanol's best-characterized effects appear after a latent period of about a day following a single (i.e., acute), large exposure, and may continue to develop or persist for days to weeks or longer.

occupational exposure to methanol vapors (Tyson. 1912: Ziegler. 1921)]. The pallor indicates a loss of the blood supply to the head of the optic nerve. and frequently reflects atrophy of the optic nerve (Casarett and Doull. 1975).

Autopsies from victims of lethal methanol poisonings have revealed gross pathology in the visceral organs. the lung. and the CNS. all of which involve a variety of edematous. hemorrhagic. and degenerative changes (Tonning. 1945: Chew et al. 1946: Province et al. 1946: Bennett et al. 1953: Kaplan. 1962: Erlanson et al. 1965). Several case studies report post-mortem signs of damage to the basal ganglia in the brain. specifically the putamen (Province et al. 1946: Erlanson et al. 1965: Aquilonius et al. 1978). This area of the brain participates in the control of gross intentional motor activities that are normally performed unconsciously. Damage to this area results in various motor disorders such as Parkinsonism and Huntington disease. A number of human studies have shown that survivors of severe methanol intoxication may suffer residual damage to the putamen and have associated motor disorders (Erlanson et al. 1965: Guggenheim et al. 1971: Aquilonius et al. 1978: Ley and Gali. 1983).

NATURE OF ACUTE TOXICITY

At this point. it is appropriate to summarize the metabolic basis of methanol toxicity. discussed in detail in the next section. Briefly. the metabolites of methanol. not methanol itself. are responsible for the toxic syndrome of acidosis and ocular toxicity that appear after the latent period (Kane et al. 1968: McMartin et al. 1980: Sejersted et al. 1983: Osterloh et al. 1986). Following uptake. methanol is metabolized in the liver to carbon dioxide (and water) through several enzymatic steps. and the carbon dioxide then is exhaled harmlessly (Tephly and McMartin. 1984). Formic acid is an intermediate product in the metabolic sequence. and. at physiologic pH. dissociates quickly and almost completely to its constituent formate and hydrogen ions. These ions then may accumulate in the body if the throughput of methanol exceeds the rate that formate is oxidized to carbon dioxide. Two related events then occur: (1) an acid load is imposed systemically. which. if persistent. eventually overwhelms acid-base homeostasis — this disorder requires roughly a day to develop and accounts for the latent period: and (2) formate exercises a localized toxic effect in the anterior region of the optic nerve by mechanisms that are still unclear.

Two facets of methanol toxicity that were appreciated quite early concern. first. the dose level of methanol that is hazardous to humans. and. second. the variable susceptibility to acute effects among individual people. Reflecting on his collection of case studies published with Buller, Wood stated in 1912:

"As in the case of several other poisons. some persons are largely immune so far as permanent damage to the organism is concerned. If ten persons drink, say, four ounces of Columbian spirits within three hours, all will have marked abdominal distress and four will die, two

of them becoming blind before death. Six will eventually recover. of whom two will be permanently blind before death. With still larger doses. the proportion of death and blindness will be greater."

In this summary statement. 4 ounces of Columbian spirits. or 95 grams of methanol (Columbian spirits is basically pure methanol) is lethal to 40% of the cases: for a 70 kg person. this dose is equivalent to about 1.4 grams of methanol per kg of body weight (g/kg). This figure is consistent with currently accepted values for lethality. and 0.3 to 1 g/kg is considered the range of a minimum lethal dose for untreated cases (Roe. 1955: Erlanson et al. 1965: Koivusalo. 1970: Gonda. et al. 1978).

As mentioned. the time to onset of symptoms among poisoned victims is quite variable. ranging from several hours to a few days. The variability of the effective dose is a prominent feature of acute methanol toxicity as well (Wood and Buller. 1904: Buller and Wood. 1904: Chew et al. 1946: Tephly and McMartin. 1984). A case report of poisoning among American soldiers in 1945 states that for each patient admitted to the hospital. up to four others had taken equivalent amounts of methanol without seeking medical care (Province et al. 1946). In the Atlanta epidemic. most patients claimed drinking about a quarter of a pint (approximately 125 cc) of "moonshine." which consisted of 40% methanol. The lowest lethal dose reported was "three teaspoons (about 15 ml)." and the largest dose survived was about a half-liter of this mixture.

Variable susceptibility is a hallmark of human and animal responses to virtually all toxic substances. The elucidation of the root causes of variability is essential to evaluate the potential public health impacts of substances likely to be ubiquitous. such as community air pollutants. Two general classes of factors determine susceptibility: metabolic and physiologic characteristics that are inherent (e.g., age. sex. genetic determinants). and the effects of external influences such as lifestyle and exposure to other substances. In the case of methanol toxicity. susceptibility factors of both classes no doubt remain unidentified. Two that are known to influence sensitivity are the amount of ethanol ingested with methanol. and the dietary sufficiency of folate.

How do ethanol and folate contribute to inter-individual variability? In humans. the hepatic enzyme that first processes methanol. alcohol dehydrogenase (ADH). also oxidizes ethanol. However. ethanol's affinity for ADH is 5 to 10 times greater than methanol's (Kini and Cooper, 1961: Makar et al. 1968). As long as ethanol persists in the circulation. it retards methanol's entrance into its metabolic pathway. thereby slowing the formation of formic acid. Thus. patients who simultaneously drink toxic doses of methanol with large amounts of ethanol. as has frequently occurred. may be spared methanol toxicity. or alternatively, may experience a delayed onset of its symptoms (Roe, 1955).

In the liver. formate is metabolized to carbon dioxide via a folate-dependent pathway. Research with animals has demonstrated that folate deficiency predisposes to the accumulation of formate, and, therefore, to a state of heightened

susceptibility to methanol toxicity (McMartin et al. 1977a; Makar and Tephly, 1977). In all likelihood, humans possess similar characteristics.

To date, modulators of susceptibility are described only for acute effects of methanol. Though these factors also may play roles in differential susceptibility to chronic effects, the list of factors that modify responses to methanol cannot be considered complete.

DEVELOPMENT OF ANIMAL MODELS

Extensive clinical and research efforts in this and other countries have been invested to understand the toxicologic, biochemical, and metabolic nature of methanol intoxication, and to improve the management of methanol-poisoned patients. In the early part of this century, the prognosis for methanol intoxication was poor, and available treatments were ineffectual. Since the 1940s, clinical advances have quickened, and the loss of vision and life from methanol exposure has abated significantly. Today, three therapeutic modes generally are used, usually in concert to alleviate the signs and symptoms of acute methanol toxicity. They are: (1) alkali treatment to restore acid-base balance, (2) ethanol treatment to retard the metabolism of methanol, and (3) hemodialysis to accelerate the clearance of methanol and formate from body fluids. (Peritoneal dialysis, less effective than hemodialysis, is also used on occasion.) Other potential treatments are still in the research or trial stages (Jacobsen and McMartin, 1986).

For the half century that followed Wood and Buller's 1904 reports, a major impediment to understand and treat methanol poisoning was the lack of awareness that only non-human primate species presented a valid model of acute human methanol toxicity. In their report on ocular effects in the Atlanta epidemic, Benton and Calhoun (1952) wrote: "It is unfortunate that laboratory animals do not respond to this agent in a manner similar to the human. Acidosis almost never develops, and the animals do not often appear to go blind."

In 1955, Roe, who in the 1940s first advocated ethanol therapy, wrote: "It is a waste of time to attempt to investigate the mechanism of the toxic effects of methanol in man by means of animal experiments until it is clear why animals do not develop more than a moderate degree of acidosis." The term "animal" in both of these quotes refers to non-primate species. Most animal research to that time focused on dogs, rabbits, rats, and mice; the few results from experiments on non-human primates did not emerge as unique. In 1955, Gilger and Potts published a landmark paper that, for the first time, established the non-human primate as the model of choice for acute methanol toxicity in humans. The paper's introduction provides an excellent review and critique of the extant literature on methanol toxicity in animals, and indicates this literature's technical inadequacies and the misconceptions it helped to create. In the experiment, Gilger and Potts (1955) exposed rats (Sprague-Dawley), rabbits, dogs, and rhesus macaque monkeys to a range of methanol doses. (They also exposed mice as reported in a previous paper (Gilger et al.

1952). Gilger and Potts observed that (1) the lethal dose for non-primates was two to three times higher than the 3 g/kg lethal dose reported for the monkeys (i.e., 6 to 10 times higher than lethal doses in humans), and (2) of all the species tested, only non-human primates experienced a sequence of early inebriation, then a one day latency followed by the toxic syndrome characteristic of humans (acidosis, some ocular toxicity), which precede their death; the other species developed an initial narcosis from which they either survived or died, and acidosis was not a prominent feature of this toxicity.

In follow-up papers, these investigators studied in non-human primates methanol-induced pathology and the effects of both bicarbonate (i.e., base) and ethanol therapy on the clinical course following lethal-if-untreated doses of methanol (Potts, 1955; Potts et al, 1955; Gilger et al, 1956; Gilger et al. 1959). (All used rhesus macaques except Gilger et al, 1959, who used both rhesus and grivet monkeys.) Both treatments effectively prevented death, as they do in humans, but bicarbonate therapy did not suppress the appearance of retinal edema. However, no signs of ocular toxicity occurred in ethanol-treated animals. These findings are consistent with the principles that (1) acidosis is the proximal cause of general symptoms and death in human methanol intoxication and (2) blocking the metabolism of methanol prevents simultaneously the acidosis and the generation of metabolites toxic to the visual system.

The legitimacy of the non-human primate model has since been confirmed (Clay et al, 1975; McMartin et al, 1975) and has enabled a systematic exploration of the metabolic bases, kinetics, and mechanisms of methanol's acute toxic syndrome, all of which are covered in greater depth in section IV. Research in lower species, primarily rats, has, nonetheless, been integral to the refinement of the non-human primate model. The contrast between the metabolic machinery of rats and non-human primates has helped pinpoint some important biochemical characteristics that influence the sensitivity or resistance to methanol poisoning. Moreover, these advances in animal models lessened the absolute reliance on human data, which are usually collected in the heat of a medical emergency. Finally, non-primates may remain appropriate models in studies that seek to understand the direct alcoholic effects of methanol.

The sub-sections that follow cover specific topics related to methanol toxicity that are relevant to the concerns of this report. Appendices are provided to amplify the discussion, when appropriate.

REPEATED OR PROLONGED HUMAN EXPOSURES TO METHANOL

As mentioned earlier, the information available suggests that extended human exposures to methanol may cause effects qualitatively similar to those from relatively high levels of acute exposure. This information is based on a limited number of case reports and even fewer epidemiologic studies. This literature, summarized in Appendix II, suffers generally from

the classic shortcomings that include unknown levels and/or durations of exposure. Nevertheless, taken together, the studies of Kingsley and Hirsch (1955), NIOSH (1981), and Frederick et al (1984), suggest that chronic exposures above 200 ppm (260 mg/m³) may produce signs of methanol toxicity, including headache, dizziness, nausea, and blurred vision. The study by Frederick et al (1984) of teacher aides who worked near spirit duplicating machines is, perhaps, the most useful study, and is reviewed in considerable detail in Appendix II. In the only no-effect study that provided ambient measurements, methanol levels were about 30 mg/m³ (Greenburg et al. 1938). For reference, the American Conference of Governmental Industrial Hygienists (ACGIH) TLV, or threshold limit value, is 260 mg/m³ time-weighted average (TWA) over 8 hours (ACGIH. 1985a); also, ACGIH has designated a short-term (15 minute) threshold value of 310 mg/m³. The ACGIH standard is reproduced in Appendix III. In 1976, the National Institute for Occupational Safety and Health (NIOSH) recommended a TWA standard of 260 mg/m³, and a 15-minute ceiling of 1,050 mg/m³ (800 ppm) (NIOSH. 1976a).

RUSSIAN STUDIES OF HUMANS: LOW-LEVEL EXPOSURES

Russian investigators published papers in 1959 and 1967 that claimed neurobehavioral effects in humans exposed to very low exposure levels of methanol vapors (less than 12 mg/m³). These are summarized here and discussed in greater detail in Appendix IV. In the first paper, Chao (1959) measured the threshold of olfactory perception and dark adaptation (or light sensitivity). The second paper by Ubaydullayev (1967), included both of these measures in addition to the EEG conditioned reflex threshold. In the olfactory studies, Chao (13 subjects) and Ubaydullayev (25 subjects) report the range for minimum detectable methanol concentrations of 4.3 to 11.1 mg/m³ and 3.7 to 10.5 mg/m³, respectively, and the range for maximum imperceptible concentrations of 3.7 to 10.5 mg/m³ and 3.9 to 9.7 mg/m³, respectively. Minimum levels of methanol that affected dark adaption were 3.3 mg/m³ for Chao (3 subjects) and 3.5 mg/m³ for Ubaydullayev (3 subjects). Finally, the latter investigator found the threshold for EEG conditioned reflexes at 1.17 mg/m³ (2 of 6 subjects).

The results of these studies suggest that exposures of several minutes or less to very low concentrations of methanol stimulate visual and peripheral olfactory receptors, and may influence the processing of stimuli in the central nervous system. The two studies, published eight years apart, produced consistent data on olfactory thresholds and qualitatively similar data on dark adaptation.

Appendix IV addresses in greater detail several problems that call the results of these studies into question. Briefly, subject descriptions were not provided, and neither were several important details concerning data acquisition — for example, no information is provided on the specific time points selected for testing dark adaptation, or on the length of exposure in the EEG conditioning test. The purity grade of methanol used was not described, which leads to a suspicion that impurities in the methanol could have confounded the determination of olfactory thresholds, and possibly the measures in the other two experiments. Also, these investigators did not discuss the manner in which methanol concentrations were measured, another potential source of error. Several features of the two dark adaptation studies display conflicts that cannot be resolved. Finally, the dark adaptation and EEG effects occurred at exposure conditions that would contribute a negligible amount to the background levels of methanol in the body (see below for discussion of background levels).

ANIMAL STUDIES SPONSORED BY NEW ENERGY DEVELOPMENT ORGANIZATION

In Japan, the Institute for Applied Energy, with the sponsorship of the New Energy Development Organization (NEDO) conducted an extensive program in which rodents and nonhuman primates (cynomolgus monkeys) were exposed to methanol vapors. (In Japan, methanol has been contemplated as a fuel for power plants.) Non-human primates were exposed chronically for up to 30 months (22 hours per day) to 13, 130, and 1,300 mg/m³ of methanol vapors. Other groups of monkeys were exposed for shorter durations (6 days to 7 months) to a higher range of levels (2,300 to 13,000 mg/m³). Also, monkeys were subjected to metabolism evaluations during a 48-hour period that followed intraperitoneal administration of methanol (25 to 3,000 mg/kg).

In the rodent experiments, rats (Fischer 344) and mice (B6C3F1) were exposed to 13, 130, and 1,300 mg/m³ of methanol for 12 months, to examine for toxic effects unrelated to carcinogenesis, and for 24 months (rats) and 18 months (mice) to examine for tumor induction (all exposures were for 20 hours per day). These same exposure levels were used in rat experiments (Sprague-Dawley) that tested for potential effects on reproductive performance over two generations. Teratology experiments also were carried out on Sprague-Dawley rats exposed to 0, 260, 1,300, and 6,500 mg/m³ for days 7 to 17 of gestation. Finally, Fischer 344 rats were subjected to metabolic evaluations like those performed on the macaques.

The NEDO program also included a battery of genotoxic assays, with various cell and bacterial systems exposed to methanol. The summary report issued by NEDO (1986) in general concludes that toxic, reproductive, and carcinogenic effects are not evident at chronic exposure levels of 130 mg/m³, and teratologic effects do not occur at 1,300 mg/m³. However, the report does indicate the possibility of subtle effects in the central nervous system of non-human primates exposed chronically to 13 mg/m³, specifically, the appearance of "reactive astrocytes." These results may or may not be of significance with regard to the exposure of the public to methanol vapors. The authors of the report attach little biological significance to these findings.

Unfortunately, the report does not include a sufficient amount of technical data and histopathological findings to

enable a critical review of the experiments and their results. However, the types of experiments performed are crucial to the evaluation of potential risks from exposure to airborne substances. Perhaps further evaluation of this program's experimental methods and data will help clarify the results observed, and their relationship to methanol exposure.

PROLONGED EXPOSURE TO NON-HUMAN PRIMATES

In a study of relevant concern, newborn stumptail macaques received aspartame in their formula daily for nine months, starting from between 17 and 42 days of age (Reynolds et al, 1984). Aspartame hydrolyzes in the gut to aspartate, phenylalanine, and methanol. The methanol accounts for 10% of aspartame's molecular weight. The exposed animals ate 1, 2, or 2.5 to 2.7 grams of aspartame/kg/day, which is the equivalent of 100, 200, and 250 to 270 mg methanol/kg/day. The investigators report no effects with respect to growth, hematology, serum chemistry, urinalysis, and EEG patterns. The investigators concluded, "Large intakes of aspartame as part of the diet appear to have no effect upon developmental parameters of the infant macaque." This experiment, however, did not include histopathologic analyses.

These same animals also were subject to hearing tests and a battery of behavioral tests that included object discrimination, pattern discrimination, and learning (Suomi, 1984). Again, the investigators were unable to detect any effects from chronic aspartame ingestion. The test battery, according to the investigators, is one "that previously had been shown to be sensitive to age difference, size and locations of cortical lesions, form of early rearing history, some chronic dietary conditions, and exposure to various environmental toxins."

In a subchronic inhalation study, cynomolgus monkeys were exposed for 4 weeks (6 hours per day, 5 days per week) to 650, 2,600 and 6,500 mg/m³ 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. Following 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. No treatment-related effects on cynomolgus monkeys were observed in this study.

PROLONGED EXPOSURE TO NON-PRIMATES

Very few studies have been conducted to investigate the potential effects of long-term exposure of non-primate species to methanol. At this point, there are no firm indications of chronic effects near exposure levels of concern. Details of the pertinent literature are in Appendix V.

FORMIC ACID

Appendix VI briefly reviews the toxicology of formic acid, which is the key toxic metabolite of methanol. The most important effects of formic acid are, apparently, those linked to its effects on the visual system, discussed in detail in Section IV.

BACKGROUND BODY BURDENS OF METHANOL AND FORMATE

Exposures to substances in the environment often contribute to a pre-existing body burden of that substance or its metabolites, found in people classified as "unexposed." For example, exposures to the airborne pollutants, carbon monoxide and manganese, contribute to background levels that would be found in people breathing clean air.

The two most prominent sources of background body burdens for methanol and formate are diet and natural metabolic processes. Sedivec et al (1981) reported a mean blood methanol level of 0.73 mg/l in 31 unexposed subjects (range: 0.32 to 2.61 mg/l), and Eriksen and Kulkarni (1963) measured a mean of 0.25 ug/l in expired breath of nine "normal" people (range: 0.06 to 0.49 ug/l). Methanol is available in the diet from eating fresh fruits and vegetables or from drinking fruit juices (average of 140 mg/l; range: 12 to 640 mg/l) and fermented beverages (up to 1.5 g/l) (Francot and Geoffroy, 1956). More recently, aspartame, an artificial sweetener, has become a part of most diets. In the gut, aspartame hydrolyzes, and 10% of its molecule, by weight, becomes free methanol that is available for absorption (Stegink, 1984). According to recent estimates, excluding carbonated beverages, daily aspartame intake will average from 3 to 11 mg/kg, with the 99th percentile ingesting up to 34 mg/kg (i.e., 3.4 mg/kg methanol) (Stegink, 1984). Carbonated beverages contain about 555 mg aspartame per liter, which means that drinking a 12-ounce (354 cc) beverage is roughly equivalent to a methanol intake of 20 mg. In addition, methanol is generated metabolically by the action of a methyltransferase enzyme system. This system methylates acceptor proteins by the action of protein carboxyl methylase, and hydrolyzes protein-methyl esters (by the action of protein methyl esterase), which releases free methanol. (Gagnon and Heisler, 1979; Stegink et al, 1983). The relative contributions of diet and metabolism to the methanol body burden has not been established.

Formate is present in the blood at background levels that range from 3 to 19 mg/l (Baumann and Angerer, 1979; Stegink et al, 1981). Formic acid is a natural ingredient of various foods such as honey (2 to 200 mg/100 g), fruit syrups (65 to 163 mg/100 g), and roasted coffee (200 to 770 mg/100 g), and also is used as a preservative (FASEB, 1976). Formic acid also participates in several metabolic processes; for example, it is a product of the metabolic degradation of several amino acids, including histidine and tryptophan, and also serves as a precursor for a variety of macromolecules (FASEB, 1976; FPF, 1982). Perhaps most germane to the present discussion, formate is a metabolite of methanol and is metabolized in the folate pathway, as discussed in detail in Section IV. The relative contributions of diet, metabolism, and methanol breakdown to the formate body burden are unknown.

The reader should bear in mind that all projections of body burdens of methanol calculated in the following section of this report reflect only the environmental contribution. For purposes of evaluation and perspective, these contributions should be compared to background levels.

IV. METABOLISM OF METHANOL AND MECHANISMS OF TOXICITY

The nature of the dose-effect relationship for any substance is rooted in the mechanisms that govern that substance's uptake and processing in the body. The better the understanding of those mechanisms, the greater one's ability to project the potential consequences that may result from an environmental exposure of a specific magnitude and time course. Furthermore, elucidating the physiologic and biochemical pathways of action furthers the understanding of the determinants of inter-individual variability and, therefore, of individual sensitivity.

This section describes the fate of inhaled methanol and its metabolites. Its objective is to provide a mechanistic and quantitative basis for methanol's acute toxic syndrome, such that one may assess the potential of anticipated ambient exposures (Section II) to initiate known toxic processes. The exposures of concern in this report, in all likelihood, result in methanol doses well below those that produce the acute effects discussed in the previous section. The literature on metabolism and toxic mechanisms, although concerned primarily with the clinical (i.e., mostly acute) aspects of poisoning, is, nevertheless, highly relevant to the objectives of this report.

Unfortunately, there are no data with which to address directly the mechanisms that underlie methanol's chronic effects. However, because the chronic effects on record (Section III and Appendix II) bear a qualitative similarity to the well-studied acute effects, one may adopt a "working" assumption that acute and chronic effects share, to some extent, common pathways of action. Of course, the possibility that chronic exposure induces effects by means presently unknown is one that must, by definition, remain open.

Section III described the early developments that led to the recognition of the non-human primate as an appropriate model for acute human methanol toxicity. Despite the unique qualities of non-human primates, there are many important characteristics of methanol's uptake and metabolism that are common to all mammalian species, and these are covered first.

UPTAKE AND DISTRIBUTION

Regardless of its exposure route, methanol distributes readily and uniformly to all organs and tissues in direct relation to their water content (Yant and Schrenk, 1937). Methanol's distribution throughout the body is, therefore, ubiquitous. Blood methanol concentration, a parameter used frequently in the literature to characterize body burden of methanol, is, on the average equal to 83% of its aqueous concen-

tration. Urine, the other body fluid most commonly sampled, contains methanol concentrations 20 to 30% higher than blood (Yant and Schrenk, 1937; Leaf and Zatman, 1952).

The means of methanol's distribution explains that all exposure routes — oral, percutaneous, intra-peritoneal, and inhalation — are equivalent toxicologically. For ingestion and intra-peritoneal administration, the initial body burden is simply the amount of methanol given divided by body weight. For short-term inhalation, an upper-bound estimate of initial body burden assumes a total absorption of the inhaled vapor. This value is calculated as $(M_v \times \dot{V}_E \times t) / BW$, where M_v = vapor concentration, $\dot{V}_E$ = ventilation rate, t = duration of exposure, BW = body weight. In reality, the absorption of inhaled methanol is less than 100%, with one reference reporting about 60% (Sedivec et al. 1981). An immediate application of this formula is to calculate the maximum initial body burden of methanol under an anticipated, worst-case, condition. Such a case might involve a 70 kg (BW) person, breathing at a rate of 20 m³/day ($\dot{V}_E$) (i.e., roughly twice resting ventilation), exposed to 200 mg/m³ (M_v) methanol vapor for 15 min (t) in a personal garage hot-soak situation (no ventilation, disabled canister). For 100% absorption, the resulting body burden is 0.6 mg/kg, which is at least 500 times lower than doses of acute clinical significance (i.e., greater than 0.3 g/kg). Table 6 lists the methanol body burdens that follow inhalation in several

Table 6. Methanol Body Burden for Selected Situations

Exposure Scenario	Condition	Added Body Burden of Methanol
Personal Garage Hot-Soak	200 mg/m³, 15 min, twice resting vent	0.6 mg/kg[a]
	100 mg/m³, 5 min, twice resting vent	0.1 mg/kg
	100 mg/m³, 5 min, resting ventilation	0.03 mg/kg
Self-Service Refueling	50 mg/m³, 4 min, twice resting vent	0.04 mg/kg
12 Ounce Diet Beverage	555 mg aspartame/l	0.3 mg/kg[b]
Dietary Intake of Aspartame (w/o diet beverages)	Normal Diet	0.3 - 1.1 mg/kg/day
"Background" Body Burden		0.5 mg/kg[c]

[a] - assumes all inhaled methanol absorbed across respiratory epithelium; in all probability, less (appx 60% of inhaled) is absorbed (Sedivec et al, 1981).

[b] - assumes all aspartame-derived methanol crosses gut mucosa instantaneously; because of the time required for hydrolysis and transport, peak measured levels reach 70-75% of the value in the table (Stegink et al. 1981).

[c] - based on value of 0.73 mg/l of blood (Sedivec et al. 1981).

scenarios of interest and those that follow dietary intake of aspartame; the table also indicates the average background body burden of methanol.

CLEARANCE AND METABOLIC KINETICS

Following uptake and distribution, methanol clears from the body. Clearance proceeds with half-times of a day or more for high doses (greater than 1 g/kg) and about three hours for low doses (less than 0.1 g/kg) (Leaf and Zatman, 1952; Kane et al. 1968; Tephly and McMartin, 1984). Methanol is either excreted unchanged (direct excretion) in urine and exhaled breath, or it enters a metabolic pathway (in the liver), whose ultimate product is carbon dioxide. The metabolic process is of importance, because methanol's acute toxic properties (Section III) are linked to intermediate metabolites, not to the alcohol itself.

The time course of methanol's disappearance from the circulation reflects the combined action of both direct excretion and metabolism. A number of studies have been conducted in humans to examine for clearance of methanol from the circulation following low-level exposures.

Leaf and Zatman (1952) monitored methanol disappearance from the circulation of three human volunteers to whom 3, 5, and 7 milliliters (or 2.4, 4.0, and 5.6 grams) of methanol were administered orally (highest dose, 0.08 g/kg). Blood levels were reflected in urine samples collected every two hours for at least 12 hours. The results were consistent for all subjects at all doses: methanol disappearance obeyed first-order kinetics with a half-time of about 3 hours (i.e., every 3 hours, the concentration of methanol was halved). See the sidebar for a description of first-order kinetics.

Sedivec et al (1981) exposed four volunteers for eight hours to methanol vapors at concentrations of 102, 205, and 300 mg/m^3. They analyzed urine samples through the exposure period and for the 18 hours that followed exposure. Urine levels of methanol were proportional to the vapor concentration throughout the observation period and, when exposure terminated, urinary methanol concentrations decreased exponentially with a half-life of roughly 2.5 to 3 hours.

Similar kinetics of methanol disappearance from blood were observed in human adults and infants to whom aspartame (10% methanol) was administered (Stegink et al, 1981; Stegink et al, 1983). The adult subjects received the equivalent of up to 20 mg/kg methanol and the infants, 10 mg/kg. Therefore, the clearance of methanol from the human circulation after body burdens as high as 80 mg/kg are achieved follows first-order kinetics with a half-time ($T_{1/2}$) of about 2.5 to 3 hours; the rate constant for total clearance, k_T, is therefore $0.693/T_{1/2}$, or between 0.23 and 0.28 hr^{-1}.

Several studies have examined the percent of methanol that is excreted as CO_2. This value reflects the fraction of the initial dose of methanol that clears by the metabolic route. The available data suggest that, following a low dose of methanol (2 mg/kg) to non-human primates (rhesus monkeys) and rats (CD-1), as much as 90% is metabolized (Opperman, 1984). Because methanol metabolism is a saturable process (see below), one expects its efficiency to decrease with larger doses. However, Eells et al (1983) showed that even after a very high

dose (1 g/kg) of radiolabeled methanol to cynomolgus monkeys, 78% of the activity recovered within 24 hours was as exhaled CO_2.

SIDEBAR

FIRST-ORDER KINETICS

A first-order clearance or metabolic process is one that proceeds at a rate proportional to the concentration of the substance undergoing that process. Expressed mathematically, $v_1 = k_1 x(t)$

where $x(t)$ = concentration of substance x at time t

v_1 = rate at which x is processed through pathway #1

k_1 = clearance constant for pathway #1

For any pathway that obeys first-order kinetics,

$x(t) = x_0 e^{-kt}$.

where x_0 = concentration of x at t = 0

and, in addition, $T_{1/2} = 0.693/k_1$, the interval required to halve the concentration of x through pathway 1 alone.

If a second first-order pathway with clearance constant k_2 is operative simultaneously, then total clearance is described by $x(t) = x_0 e^{-(k_1 + k_2)t}$. Further, pathway #1 is responsible for $k_1/(k_1 + k_2)$ of the total cleared and #2 for $k_2/(k_1 + k_2)$ of the total. For three pathways, the rate constant is $k_1 + k_2 + k_3$, pathway #1 metabolizes $k_1/(k_1 + k_2 + k_3)$ of the total, and so on.

MICHAELIS-MENTEN KINETICS

In a reaction that obeys Michaelis-Menten kinetics, v, the rate of that reaction (e.g., metabolic conversion), is related to the concentration of the reactant (in this case, methanol) as follows:

$$v = V_{max} \frac{x}{K_M + x}$$

where, V_{max} = maximum velocity of reaction as x approaches infinity and,

K_M = the Michaelis constant, which defines the concentration at which

$v = \frac{1}{2} V_{max}$

The relationship, drawn in the figure, reflects that at concentrations large compared to K_M, the reaction approaches saturation. In other words, it operates with nearly zero-order kinetics, which means that v is nearly independent of x. At low concentrations that are small compared to K_M, $v = (V_{max}/K_M)x$. Such reactions are first-order because the reaction velocity is related almost linearly to concentration or $v = kx$ and $k = V_{max}/K_M$.
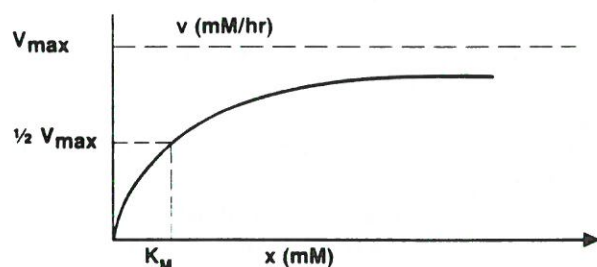
MECHANISMS OF CLEARANCE

The discussion that follows develops in greater detail the mechanisms that account for metabolism's dominant role in methanol clearance. This analysis will enable a sharper assessment of the potential for health effects under expected exposure conditions.

The observations that total clearance of methanol from body water proceeds with first-order kinetics at low doses (less than 0.1 g/kg) justifies the supposition that each separate pathway also obeys first-order kinetics, and is, thus, characterized uniquely by its own rate constant (see sidebar). If such is the case, k_T, the rate constant for total clearance, equals the sum of rate constants for all pathways; further, the fractional contribution of each pathway to clearance is equal to its rate constant divided by the sum of constants.

Each of the three major clearance pathways that were identified earlier may be assigned a rate constant: k_r represents direct renal excretion of methanol; k_p represents direct pulmonary excretion of methanol; and k_m represents metabolic clearance. Expressed mathematically, the preceding states that, if all pathways obey first-order kinetics, then, $k_T = k_r + k_p + k_m$, and that the fractional contribution of each pathway, for example, metabolism, is k_m/k_T. These pathways are displayed schematically in Figure 2. The figure indicates with a dotted line that, in addition to CO_2, the metabolic pathway generates formate, which is detectable in urine. Formate is the toxic metabolite apparently responsible for methanol's acute toxic syndrome in humans and primates. However, at the low doses of concern in this report, formate, as a metabolic end product, is excreted in quantities that are negligible compared to CO_2. Thus, for the analysis presented in the discussion that follows, CO_2 excretion represents the quantitative metabolism of methanol. The principles governing formate generation are explored, in depth, later in this section.

In the following analysis, rate constants for each pathway are derived on the basis of physiologic or kinetic principles, and, to test their validity, they are compared to empirical data:

(1) Direct renal excretion of methanol: The kidney apparently exerts no active control over the urinary concentration of methanol. Consequently, the methanol content of urine that enters the bladder maintains the aqueous concentration of methanol in the blood that entered the kidney (Yant and Schrenk, 1937; Leaf and Zatman, 1952; Sedivec et al. 1981). Thus, the rate at which methanol clears into the urine is directly proportional to its blood level, which satisfies the condition for first-order kinetics.

Under these conditions, k_r, the rate constant for direct renal excretion of methanol, equals the rate of urine formation divided by the total volume of body water. A "typical" 70 kg person contains roughly 42 liters of water (60%) and produces on the average 60 milliliters (ml) of urine per hour (0.86 ml/hr/kg). Therefore, one may project a k_r of 0.0014 hr^{-1}; half-time for this pathway is simply $0.693/k_r$ (20 days). This simple formulation allows one to predict that renal processes account for k_r/k_T of all the methanol cleared. Since k_T was estimated earlier as 0.23, the renal contribution to the total is about 0.6%.

Leaf and Zatman (1952) measured urinary excretion of methanol from three human subjects after they ingested a dose of 3.2 g of methanol (avg 0.05 g/kg). These investigators determined that at 12 hours post-ingestion (when more than 90% of a methanol dose has cleared the circulation), an average of 0.76% of the initial dose was excreted in the urine. Jacobsen et al (1983) studied renal clearance of methanol in a 65 kg victim of methanol poisoning who was undergoing treatment, which included diuresis of 7.0 liters over a 24 hour period, i.e., a urine production of 4.2 ml/hr/kg; as mentioned above, urine formation in a "normal" 70 kg person proceeds at around 60 ml/hr, or 0.86 ml/hr/kg. Renal clearance of methanol in the patient was measured as 5.7 ml/min (0.34 l/hr). (Clearance expressed in this manner has only a hypothetical meaning — it is as if each minute, 5.7 ml of blood were totally cleared of methanol.) Assuming the patient had a body water content of 60%, and a blood water content of 83%, the calculated
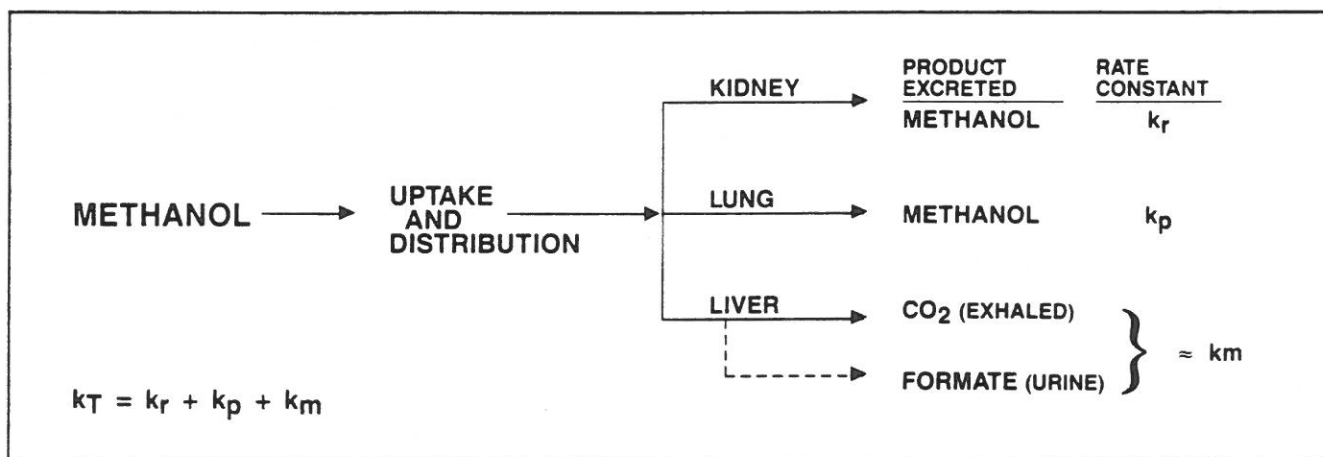

Figure 2. Schematic Diagram of Clearance Pathways for Methanol Following Uptake and Distribution Throughout the Body.

rate constant for renal clearance of methanol during diuresis is. (0.34 l/hr x 0.83)/(65 kg x 0.6 l/kg) = 0.007 hr⁻¹.

The value just calculated was obtained from the study of a patient treated with diuresis. To obtain a renal clearance constant (k_r) for a person who is producing "normal" quantities of urine requires multiplying the clearance value just calculated by the ratio of "normal" urine production (0.86 ml/hr/kg) to urine production in the patient (4.2 ml/hr/kg), which yields a k_r of 0.0014 hr⁻¹. On the basis of these data and calculations. the above estimate of k_r seems reasonable.

(2) Direct pulmonary excretion of methanol: In the lung, a small fraction of blood-borne methanol diffuses passively from the pulmonary capillaries to the alveoli and is exhaled. The amount of methanol that crosses the blood-air barrier is directly proportional to its blood concentration — i.e., first-order kinetics — and is governed by its blood-air partition ratio: this parameter describes the relative content. at equilibrium. of a substance in each of two phases in contact.

For a first-order process. the rate of pulmonary excretion of methanol = k_p x blood methanol concentration. The rate constant. k_p. may be approximated by $V_A/(PR \times bw)$ where.

V_A = alveolar ventilation. approx 500 liters/hour during moderate activity for a typical 70 kg person.

bw = body water. 42 liters for same person.

PR = methanol's blood-air partition ratio. approximately 2.000 (Harger et al. 1950).

Plugging in the above values yields a value for k_p of 0.006 hr⁻¹ (and therefore. a 5 day half-time). This derivation of k_p's hypothetical value is based. for purposes of simplification. on a constant alveolar ventilation. However. altering ventilation will affect the rate at which methanol is exhaled.

Jacobsen et al (1983) also studied pulmonary excretion of methanol in the 65 kg patient described above. The patient. though admitted with methanol poisoning, was not hyperventilating. These investigators calculated a pulmonary clearance rate of 5.6 ml of blood per minute (0.34 l/hr) (i.e., each minute. 5.6 ml of blood is hypothetically cleared totally of methanol). Assuming the patient had a body water content of 60% and a blood water content of 83%, the calculated rate constant k_p equals (0.34 l/hr x 0.83)/(65 kg x 0.6 l/kg) = 0.007 hr⁻¹. This value and the theoretical estimate for k_p (0.006 hr⁻¹) are quite consistent.

(3) Metabolic clearance: In contrast to direct renal and pulmonary excretion. the metabolic conversion of methanol to carbon dioxide is not linear with concentration. In the 1960s, Tephly and colleagues demonstrated, in both rats (Holtzman) and rhesus monkeys, that in vivo metabolism of methanol to CO_2 obeys Michaelis-Menten kinetics (see sidebar) and, further. that the metabolic kinetics for both species are very similar (Table 7) (Tephly et al. 1964; Makar et al. 1968). Briefly, this means that with rising blood concentrations of methanol, the metabolic pathway demonstrates saturability, and approaches a maximal conversion rate that it is unable to exceed. At saturation, the pathway demonstrates zero-order

kinetics. which means the metabolic conversion rate is independent of concentration. However. at low concentrations — much lower than the Michaelis constant. K_M — processes that obey Michaelis-Menten kinetics behave as if they are first-order processes. with a rate constant. k_m of $V_{max} \cdot K_M$ (explained in sidebar). Subsequent time-course observations (Noker et al. 1980) of methanol clearance from the blood of cynomolgus monkeys given 2 g/kg of methanol provide data that are consistent with those on rhesus monkeys shown in Table 7; specifically. Noker et al (1980) recorded a zero-order clearance of about 50 mg/kg/hr, and their graphic data display a transition from zero to first-order kinetics at around 10 millimoles of methanol per liter of blood or 12 mM aqueous concentration.

Table 7. "In Vivo" Michaelis-Menten Constants for the Metabolic Conversion of C^{14}-Methanol to $C^{14}O_2$

Species	V_{max} (mg/kg/hr)	K_m (mM)
Non-human Primates[1] (rhesus monkeys)	48	8.7
Rats[2]	30	7.0

[1] - Makar et al. 1968
[2] - Tephly et al. 1964

As estimated earlier in this section. a worst-case single exposure (200 mg/m₃ methanol for 15 minutes) produces a methanol body burden of less than 1 mg/kg. For an individual with 60% body water. this upper level burden corresponds to 0.05 millimoles of methanol per liter (mM). This concentration is less than 1% of the K_M for monkeys (roughly 10 mM), which reinforces the notion that. for environmental exposures of interest in this report. metabolism is operating in a first-order domain. To calculate k_m requires transforming V_{max} into units of mM/hr. Makar et al (1968) used carbon-14 labeled methanol (MW 34), and estimated body water of rhesus monkeys at 70% of their weight. Thus. based on the values for rhesus monkeys in Table 7.

$$k_m = \frac{V_{max}}{K_M} = \frac{1}{8.7} \times \frac{48}{(34)(0.7)} = 0.23 \text{ hr}^{-1}$$

The metabolic rate constant derived here is clearly much greater than the sum of constants for both direct renal (0.0014 hr⁻¹) and direct pulmonary (0.0060 hr⁻¹) excretion, which explains. from a kinetic viewpoint. why metabolism is expected to dominate the clearance of methanol from body water. As indicated earlier, this dominance has been confirmed in animal experiments. Table 8 summarizes the preceding analyses, and demonstrates that the clearance patterns of methanol observed empirically are consistent with those one would project using basic physiologic principles and Michaelis-Menten constants derived from studies of non-human primates.

Table 8. Contribution of Renal, Pulmonary, and Metabolic Pathways to the Overall Clearance of Low Doses of Methanol (< 0.1 g/kg) : Comparison of Observed and Derived Rate Constants

| Clearance Pathway | Derived Values | | | Exptl/Clin Observations | Source |
	Rate Constant k (hr^{-1})	Percent Clearance	Source		
Direct Renal	0.0014	0.6	Basic Considerations	0.76% of initial dose excreted in urine by 12 hours post-ingestion	Leaf and Zatman, 1952
				k_r of 0.0014 hr^{-1} for "normal" derived from patient on diuresis	Jacobsen et al, 1983
Direct Pulmonary	0.0060	2.5	Basic Considerations (Partition ratio from Harger et al. 1950)	k_p of 0.007 hr^{-1} derived from patient data	Jacobsen et al. 1983
Metabolic	0.23	96.9	Makar et al. 1968	90% or more of dose excreted as CO_2	Opperman, 1984
Total	0.24	100.0		half-time of approximately 2.5 to 3 hours observed in humans, i.e., $k_T = 0.23$-0.28	Leaf and Zatman, 1952 Sedivec et al. 1981 Stegink et al. 1981 Stegink et al. 1983

TOXIC MECHANISMS AND SPECIES SENSITIVITY

The above discussion dealt with metabolism as if it proceeded as a "black-box" process with input (methanol) and output (CO_2). This subsection probes the workings of that "black box," and describes the biochemical events within the metabolic pathway that trigger methanol's toxic response, and discusses the major factors that influence species and individual susceptibility. The mechanisms discussed are associated directly with acute effects, as laboratory experiments and clinical analyses have amply demonstrated, but they probably contribute to chronic effects as well. In the liver, the sequence of intermediate metabolites that lead from methanol to carbon dioxide is the same in all mammalian species studied (Tephly and McMartin, 1984):

$$\text{Methanol} \xrightarrow{1} \text{Formaldehyde} \xrightarrow{2} \underset{(+H^+)}{\text{Formate}} \xrightarrow{3} CO_2 + H_2O$$
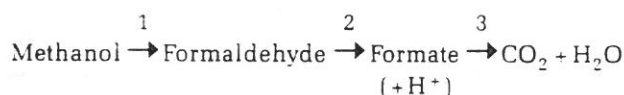
Although the sequence of metabolites is identical for all species, there are interspecies differences with respect to the details of the reactions. Several of these differences bear directly on sensitivity to methanol and several appear not to. Appendix VII, excerpted from Tephly and McMartin (1984), describes in depth the details of methanol metabolism in both non-human primates and non-primates. The discussion that follows briefly reviews the entire metabolic process, but deals mainly with the aspects of metabolism related to toxicity.

The toxic properties of methanol, and the basis of species susceptibility, are rooted in the factors that govern the relative rates of formic acid (formate + H^+) generation (steps 1 and 2) and formate oxidation to CO_2 (step 3). In short, the toxic

syndrome sets in if formate generation continues at a rate that exceeds its rate of removal; the latter is a mainly a function of metabolism to CO_2, as renal excretion of formate plays a minor role. This imbalance, if protracted, leads to an accumulation of formate coupled, eventually, to an uncompensated metabolic acidosis. The acidosis, if untreated, can prove lethal; however, even at physiologic pH, formate is associated with ocular toxicity. Rats dispose of formate efficiently at any dose and thus escape toxicity, whereas, at sufficiently high doses, humans and non-human primates accumulate toxic metabolites and do not fare as well. The key to species differences lies in step 3 and, as explained below, it is directly related to the maximum rate at which the liver oxidizes formate.

Step 1: The first step in the metabolic sequence, in which methanol is oxidized to formaldehyde, reflects the major route of methanol clearance from the circulation (see previous discussion). In non-human primates, alcohol dehydrogenase mediates this initial step (rhesus monkeys, McMartin et al, 1975); in rats, a catalase-peroxidative system is primarily responsible (Tephly et al, 1964 - Holtzman strain; Makar and Mannering, 1968 - Sprague-Dawley). Despite this difference, the initial metabolic step proceeds at similar rates in non-human primates and rats (Tephly et al, 1964; Makar et al, 1968; Tephly and McMartin, 1984). The values for V_{max} in Table 7 represent, fairly well, the maximal clearance rates observed in both species, although several studies in non-human primates have displayed even higher rates (up to 62 mg/kg/hr) (Bartlett, 1950; McMartin et al, 1975; Noker et al, 1980; Eells et al, 1983). The rate at which methanol disappears from the circulation and enters its metabolic process is independent

of "downstream" manipulations that either stimulate or slow the oxidation of formate (step 3) (Eells et al. 1983).

Step 2: The second metabolic step converts formaldehyde to formic acid, which, in turn, dissociates to formate and a hydrogen ion. The step occurs as a two-reaction process, the second of which is irreversible. The first reaction, in which formaldehyde is oxidized to S-formylglutathione, requires reduced glutathione (GSH), and is mediated by an NAD-dependent formaldehyde dehydrogenase. In the second reaction, thiolase catalyzes the conversion of S-formylglutathione to formic acid (Tephly and McMartin, 1984).

The nature of formaldehyde oxidation is important because formaldehyde itself is potentially toxic, and several "modern" references ascribe methanol's ocular toxicity to it (Loomis, 1968; Casarett and Doull, 1975). Formaldehyde has not, however, been detected in body fluids or tissues following toxic methanol exposures (Makar and Tephly, 1977; McMartin et al. 1977b; McMartin et al. 1979). Furthermore, its clearance from the bloodstream following intravenous infusion occurs with a half-life of between one and one-and-a-half minutes, and is followed by an immediate and corresponding rise in blood formate (Malorny et al. 1965; Rietbrock, 1965; McMartin et al. 1979). The possibility that formaldehyde generated in the liver is active in the optic tract seems unlikely. Martin-Amat et al (1977) have demonstrated, moreover, that formate alone given intravenously to rhesus monkeys induces the same ocular toxicity that is characteristic of methanol poisoning, with no sign of formaldehyde present.

Thus, evidence supporting a role for formaldehyde in methanol toxicity is still lacking. The possibility that formaldehyde is generated locally in the retina or proximal optic nerve has not yet been ruled out, and may merit some further attention. However, the present focus on formate as the principal toxic agent appears justified.

Step 3: In both non-human primates and rats, a folate-dependent pathway in the liver is responsible for the reactions that metabolize formate (step 3). Figure 3 shows the major aspects of this pathway. Formate first forms a complex with tetrahydrofolate (THF) that is sequentially converted to 10-formyl-THF (by formyl-THF synthetase) and then to carbon dioxide (by formyl-THF dehydrogenase). THF is derived from folic acid in the diet and also is regenerated in the folate pathway.

Although the folate pathway metabolizes formate in both species, rats use the pathway more efficiently, a fact that lies at the heart of species sensitivity to methanol. Both formate clearance from the blood and its metabolism to CO_2 proceed about 2 to 2½ times faster in rats than in non-human primates. For example, Clay et al (1975) showed that, following its intravenous infusion at doses lower than 100 mg/kg (2.2 mmole/kg), formate clears from rats with a half-time of 12 minutes, and from non-human primates (pigtail macaque) with a half-time of 31 minutes, and that the respective half-times decrease with increasing dose in both species, which indicates that formate metabolism is a saturable process; these
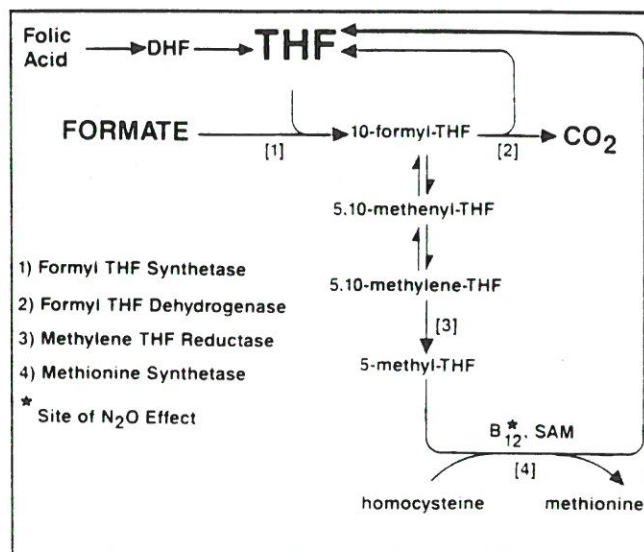
Figure 3: Metabolism of formate by folate-dependent reactions. DHF, dihydrofolic acid; THF, tetrahydrofolic acid; SAM, S-adenosylmethionine. Reaction 1 is catalyzed by formyl-THF synthetase and requires prior activation of formate with ATP. Reaction 2 involves [¹⁰N] formyl-THF dehydrogenase and utilizes NADP as a hydrogen acceptor. Reaction 3 is catalyzed by 5,10-methylene-THF reductase and is essentially irreversible. Reaction 4 is catalyzed by 5-methyl-THF homocysteine methyltransferase (methionine synthetase) and requires catalytic amounts of vitamin B₁₂ and SAM.

findings are consistent with previous research (Malorny, 1969a; Rietbrock, 1969). Specifically, in the study by Clay et al (1975), half-times in pigtail monkeys were 51 minutes at 470 mg/kg, 49 minutes at 285 mg/kg, 46 minutes at 200 mg/kg and 31 minutes at both 72 and 50 mg/kg; at 670 mg/kg, formate half-time in rats was 23 minutes. However, following high doses of methanol (4 g/kg in pigtail monkeys, 6 g/kg in Sprague-Dawley rats) formate accumulates appreciably only in monkeys, with only a subtle increase seen in rats (Figure 4) (Clay et al, 1975). On the basis of relative metabolic efficiencies, one may have expected rats to accumulate 40 to 50% of the formate measured in non-human primates.

The apparent reason why rats do not accumulate formate noticeably at any dose of methanol is that the maximal rate of formate oxidation in rats (1.6 mmole/kg/hr) exceeds the maximal rate that steps 1 and 2 supply substrate (from Table 7, 30 mg/kg/hr, equivalent to 0.9 mmole/kg/hr). On the other hand, in non-human primates, the "upstream" processes are able, with high enough methanol concentrations, to feed formate to the folate pathway at a rate (1.4 to 1.8 mmole/kg/hr) that exceeds the pathway's metabolic capacity (0.75 mmole/kg/hr). The "spillover" accumulates in the circulation which, if protracted, leads to the toxic consequences of methanol poisoning. These relationships are shown in Figure 5. At low doses of methanol, the process that clears methanol from the bloodstream and generates formate (3 hour half-time) is considerably less efficient than the process that clears formate (half-hour half-time in primates). Therefore, at low methanol expo-

sures. formate is not expected to accumulate to any major degree. Baumann and Angerer (1979) report that 20 workers exposed to an average of 140 mg/m^3 methanol for an 8-hour work day registered an increased blood formate of 0.1 mM at the end of their work-shift (background for humans ranges from 0.07 to 0.4 mM — see Section III). Urinary excretion of formate, though detectable following both human and animal methanol exposures, plays a minor role relative to metabolism in the overall clearance of formate.
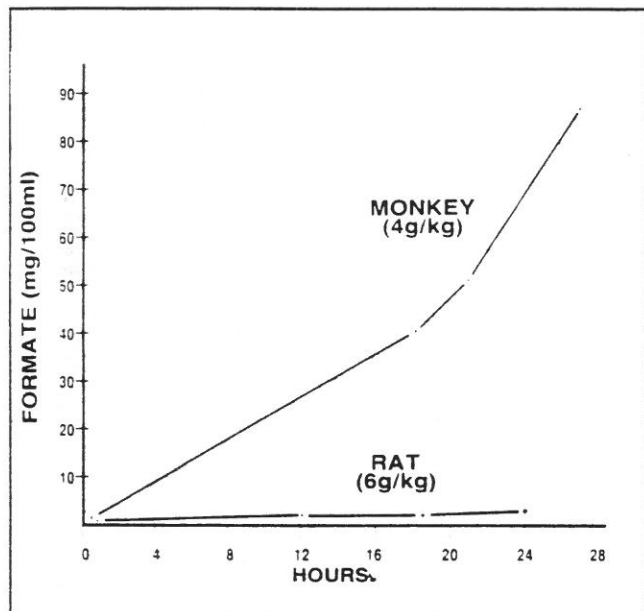
Figure 4: Blood formate concentrations in the monkey and rat after administration of methanol. Methanol was administered as a 25% solution in saline to the monkey (4 g/kg, ip) and the rat (6 g/kg, ip). Blood specimens were drawn at the indicated times after methanol administration.
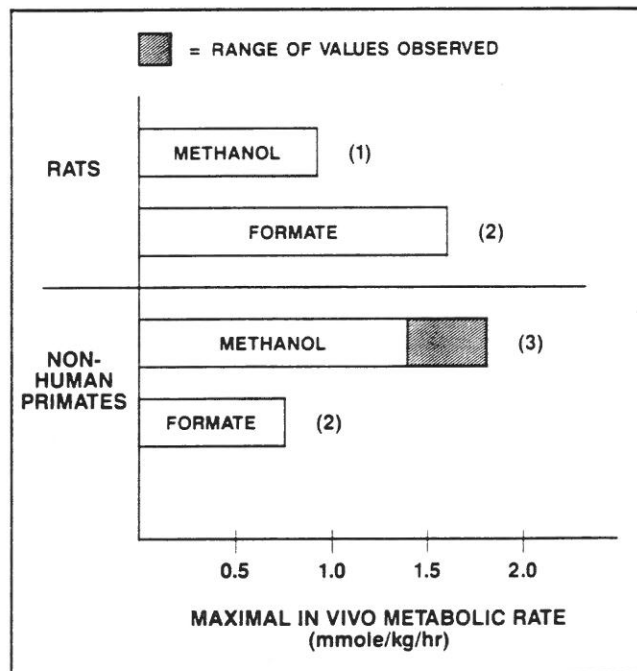
Source: Clay et al. 1975
Reprinted, by permission, from Academic Press.

The efficiency of formate metabolism is linked strongly to the hepatic concentration of tetrahydrofolate (THF), the molecule that initially complexes with formate. The factors, in turn, that control THF levels involve both the dietary level of folate and the dynamic equilibrium within the biochemical loop that regenerates THF (Figure 3).

In the 1970s, Tephly and colleagues studied the effects of dietary folate on species susceptibility to methanol toxicity. Makar and Tephly (1976) fed rats (Sprague-Dawley) a folate deficient diet, and observed that these subjects suffer the effects of a methanol dose (4 g/kg) that such animals ordinarily tolerate well when maintained on a control diet. (The folate-deficient period, though not specified in Makar and Tephly (1976), was probably similar to the 6 to 8 week period used by Palese and Tephly (1975).) Specifically, folate-deficient rats became acidotic and accumulated formate (but not formaldehyde) in the same manner observed previously in non-human primates (Makar and Tephly, 1976, 1977). The key to methanol toxicity in folate-deficient rats is a diminished

capacity to oxidize formate (Palese and Tephly, 1975; Makar and Tephly, 1977). On the other hand, cynomolgus monkeys treated with folate or a folate derivative (5-formyl-THF), and given 2 g/kg of methanol, maintained normal blood pH associated with only a modest rise in blood formate (Noker et al. 1980); control monkeys experienced a larger rise in formate and became temporarily acidotic. As one would expect, folate-deficient monkeys displayed even greater susceptibility to methanol than their normally fed counterparts (McMartin et al. 1977a). In all of these cases, the effect of dietary folate on formate metabolism was the direct link to altered sensitivity to methanol; slowing formate metabolism induced a methanol-sensitive state.

The dependence of formate oxidation on endogenous folate regeneration has been demonstrated in both rats and non-human primates. Using both species, Eells and colleagues performed a series of experiments in which the folate feedback loop was opened pharmacologically and the animals then were challenged with either formate or methanol (Eells et al. 1981; Eells et al. 1982; Eells et al. 1983). The agent used to poison the loop was nitrous oxide (N_2O), an anesthetic gas, which, among its actions, blocks methionine synthetase (Figure 3, Step 4) by inactivating vitamin B_{12}, the enzyme's cofactor. Nitrous oxide treatment slowed formate metabolism and heightened methanol sensitivity. With N_2O, rats (Sprague-Dawley) given 4g/kg methanol and monkeys (cynomolgus), given 1 g/kg became acidotic, but animals given methanol without N_2O maintained normal blood pH.

Figure 5: Maximal In Vivo Metabolic Rates for Methanol and Formate in the Rat and Non-human Primate

Source: (1) Tephly et al. 1964
(2) McMartin et al. 1977a
(3) Makar et al. 1968, McMartin et al. 1975, Noker et al. 1980, Eells et al. 1983

Eells et al (1982) further showed. using Sprague-Dawley rats. that the critical variable in these experiments was the concentration of hepatic THF. The concentration of total hepatic folate remained unaffected by N_2O treatment. The extent to which N_2O slowed formate oxidation was linked directly to the extent that N_2O decreased hepatic levels of THF. When plotted against each other. formate oxidation and hepatic THF correlated very closely (r = 0.89). Nitrous oxide thus acts to upset the dynamic equilibrium of the folate regenerative pathway so as to increase methylated and formylated folates at the expense of THF.

Subsequent research has focused on the comparative aspects of folate biochemistry among rats. monkeys, and humans. Black et al (1985) studied rats (Sprague-Dawley) and non-human primates (cynomolgus monkeys) and compared the levels of folate intermediates and the activities of folate-dependent enzymes in the livers of both species. Though total folate content of the two was practically identical. the THF concentration in monkey livers was 59% of the value in rats. This lower level of THF in non-human primates is consistent with their heightened sensitivity to methanol. and the ratio of THF levels in the two species is similar to the ratio of their maximal rates of formate oxidation (47%). The authors describe several species differences in folate-dependent enzyme activities that may contribute to different states of dynamic equilibrium in the folate regenerative pathway, whereby the species-specific balance of THF to total folate favors the rat's natural resistance to methanol. The authors note that the equivalence of total folate in both species "suggests that monkeys do not have a deficit in the dietary or hepatic uptake of folates. as compared to rats."

More recently. Johlin et al (1986) reported that human levels of total hepatic folate and THF were 60% and 50% of the respective levels in rat liver. Research is continuing to unravel the intricacies of the folate pathway and its relationship with methanol toxicity.

## SEQUENCE OF ACUTE TOXICITY: ACIDOSIS AND OCULAR TOXICITY

The establishment and refinement of the non-human primate model has enabled researchers to both characterize carefully the sequence of events that follow large single doses of methanol. and to propose mechanisms of action. Figure 6 from Tephly (1977) illustrates the time course for several key blood parameters that follows a lethal (3 g/kg) dose of methanol. As shown. in the symptomless latent period. formate accumulates and homeostatic mechanisms compensate for the increasing acid load. Between 12 and 16 hours post-ingestion. homeostatic reserve is exhausted, and the animal enters an acidosis from which it does not recover. Through the latter phase. the blood level of formate (and perhaps of other organic ions such as lactate) continues to increase. With lower doses (0.5 g/kg and 2.0 g/kg) formate and acid start to accumulate, but. with a continuously diminishing supply of methanol in the bloodstream. the critical blood parameters

(formate. pH) revert to normal values (Figure 7). Presumably. at these lower doses. the initial steps that oxidize methanol generate formate in excess of the folate pathway's formate-metabolizing capacity. However. as methanol clearance continues. and its blood concentration drops. the rate of formate generation drops below the folate pathway's capacity. which allows both the throughput of formate and the excess blood formate to be metabolized. and results in a return to normal. For lethal doses. formate generation continues in excess of the formate-metabolizing capacity into the terminal state.

Non-human primates and humans that survive the lethal effects of methanol are still at considerable risk of developing damage to the visual system. In 1977. Martin-Amat et al introduced a non-human primate model (rhesus monkeys) for sustaining methanol toxicity at a sub-lethal level to allow full expression of the ocular toxicity to develop: acidosis and elevated formate in blood (and cerebrospinal fluid) characterized this sustained toxic period. Using this model. Hayreh et al (1977) noted ophthalmoscopic changes. consisting primarily of hyperemia and edema of the optic disc. between 43 and 171 hours from the start of ingestion. Except for some engorgement of the retinal veins in the later phase of edema. the retinal vasculature remained normal throughout the observation period. Optic disk edema was the only sign of angiographic abnormality in the entire retina. In addition. the monkeys' pupils were dilated and unresponsive to light. as with human intoxication.

Baumbach et al (1977) further explored the injury to the visual system using light and electron microscopic examinations of tissue from the same animals that Hayreh et al studied. According to their observations. the primary sites of injury are the optic nerve head and the intraorbital portion of the optic nerve (i.e., the site where the retinal nerve fibers converge and become a nerve bundle); the retina itself is undisturbed. They state. "The morphological alterations seen in the optic nerve and optic nerve head in methyl alcohol-poisoned rhesus monkeys can be separated into two categories — alterations within axons and alterations of glial cells. Alterations seen in axons include mitochondrial swelling and clustering, neurotubular disruption. the formation of vesicles. and increased density of amorphous proteins. and axonal enlargement. Glial cell alterations include astrocytic swelling and swelling of the oligodendroglial cytoplasm in contact with the optic nerve axon."

The precise mechanisms of this injury are not yet clear. but several investigators have suggested a sequence that involves the interaction of formate-induced metabolic inhibition with the observed compression and swelling of optic nerve fibers. The initiating event is believed to involve formate's action as a metabolic poison. specifically as an inhibitor of cytochrome oxidase. This protein is located in the mitochondria and is the last in a chain of cytochromes and other molecules that transfer electrons sequentially to molecular oxygen. In so doing, they generate adenosine triphosphate (ATP), the cell's basic molecular form of energy. Formate inhibits cytochrome
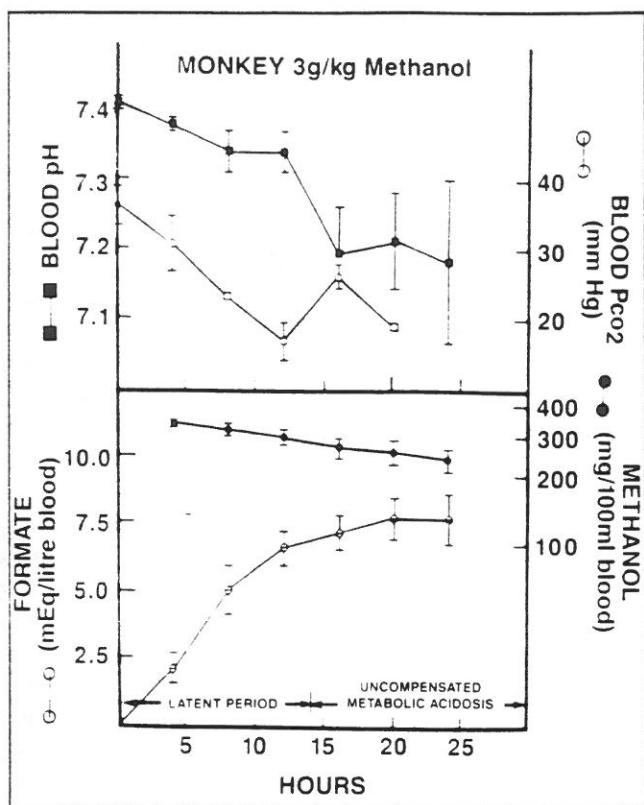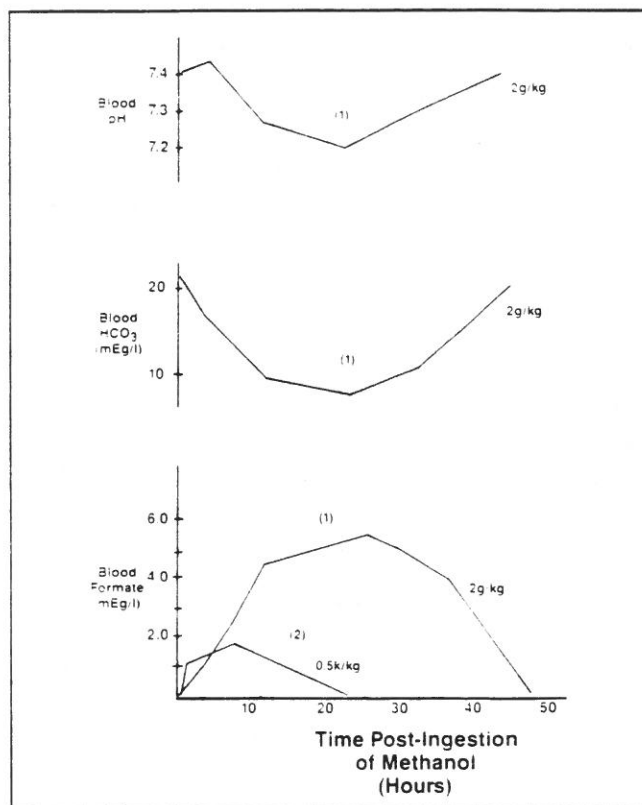
Figure 6: Metabolic acidosis, formate accumulation, and methanol metabolism in the monkey. Each point represents the mean ± SEM of at least three animals.

Source: Tephly, 1977

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Figure 7: Blood levels of bicarbonate and formate and blood pH following oral administration of methanol to cynomolgus monkeys
Adapted from: (1) Noker et al. 1980
(2) McMartin et al. 1977a

oxidase with a K_i (the concentration that achieves 50% cytochrome inhibition) of between 5 and 30 mM, which overlaps the range of formate concentrations observed in acute methanol intoxication both experimentally and clinically (Nicholls 1975,1976). Furthermore, as pH decreases, inhibition increases, which suggests that the active species is undissociated formic acid (described further below).

The myelin sheath (or white matter) of the optic nerve is comprised of cells that normally have low reserves of cytochrome oxidase, owing to their low metabolic requirement, and thus may be particularly sensitive to formate-induced metabolic inhibition. At the point where the optic nerve bundle forms, formate has access to the nerve from both the choroid circulation behind the retina and from the cerebrospinal fluid. [Martin-Amat et al (1977) observed formate accumulation in the cerebrospinal fluid of rhesus monkeys given large methanol doses; the CSF formate levels were similar to those in the blood.] If sufficiently damaged from metabolic inhibition, the cells of the white matter (oligodendroglial cells) may swell and, in essence, form small "cuffs" around individual nerve fibers, which themselves may then exhibit a compression-type injury in the form of swelling and edema proximal to the cuff in the optic nerve head. Such compression on the fiber will slow the transport of proteins, neurotubules, and mitochondria from the fiber's cell body (located

in the retina — the retinal ganglion cell) to the fiber axoplasm which renders the fiber increasingly deficient in essentials and, thus, susceptible to formate-induced injury. Once the nerve fiber is no longer able to sustain the metabolic energy to conduct electrical impulses, visual decrements will occur. This schema for the progression of toxic events is consistent with the morphological evidence presented by Hayreh et al (1977) and Baumbach et al (1977), who used the non-human primate model for methanol-induced ocular toxicity.

The onset of acidosis may serve to accelerate the sequence just described. As mentioned, the inhibition of cytochrome oxidase increases with decreasing pH, which suggests that undissociated formic acid is the active inhibitor of cytochrome oxidase. The Henderson-Hasselbalch equation predicts that with a pH drop of 0.3, which is commonly observed in methanol poisoning, the concentration of undissociated formic acid (pK_a 3.8) doubles. Thus, acidosis may potentiate the biochemical inhibition of cellular respiration, and speed the onset of cellular injury. Also, the acidosis as it progresses will begin to induce circulatory failure, which leads to tissue hypoxia and lactate production, both of which further increase the acid load, increasing undissociated formic acid, etc. This cycle, termed "circulus hypoxicus" (Figure 8) by Jacobsen and McMartin (1986), may hasten the end stage consequences of methanol poisoning. While acidosis may accelerate formate

toxicity. Martin-Amat et al (1978) showed that, even at physiologic pH, formate is capable of inducing the same ocular toxicity in non-human primates (rhesus monkeys) as is observed in methanol poisoning. Formate maintained between 12 and 34 mEq/l (equivalent to mM) in the blood for 25 to 50 hours inhibited the pupillary response and caused optic disc edema.

ACCUMULATION OF FORMATE IN HUMANS: HIGH AND LOW DOSES

Large increases in circulating formate are linked firmly to the acute toxic manifestations that result from large doses of methanol (approximately 0.3 g/kg and above). As discussed earlier in this section, one would expect a sharp increase in formate accumulation to occur with saturation of the folate pathway, which metabolizes formate. Applying formulas and values used previously in this report, and assuming that non-human primates adequately model humans, one may calculate the approximate dose of methanol that achieves this saturation. Assume (1) the maximal rate of methanol metabolism (V_{max}) in humans is equivalent to the value given in Table 7 for non-human primates, 48 mg C^{14}-methanol/kg/hr (or 2.0 mM/hr for a non-human primate that consists of 70% water), (2) the maximal rate of formate oxidation is the same for both species, 35 mg C^{14}-formate/kg/hr or 1.1 mM/hr, (3) the Michaelis constant for methanol metabolism in humans is the same as in non-human primates, 8.7 mM, and (4) formate is relatively evenly distributed through body water, a reasonable assumption based on the determination by Jacobsen et al (1983) of formate's volume of distribution (0.5 liters/kg). Then using the Michaelis-Menten equation, one can estimate the concentration (or dose) of methanol, M, that puts the folate pathway into saturation, as follows:

$$1.1 = 2.0 \times \frac{M}{8.7 + M} \quad : M = 11 \text{ mM or 210 mg/kg, for a person with 60\% body water}$$

The calculated value of M, though only a crude estimate, is not far from the low end of the range of methanol doses considered potentially significant clinically (0.3 g/kg). For reference to the situations of concern in this report, a 210 mg/kg body burden of methanol would be achieved if a 70 kg person breathing at 20 m^3/day (twice resting) was exposed for an hour to roughly 18,000 mg/m^3, absorbing 100% of the material inhaled. Since worst-case conditions for single exposures present about 200 mg/m^3 for the period spent in a hot-soak garage (15 minutes or less), the likelihood of overwhelming the folate pathway in such situations seems remote. The same conclusion also would probably apply to attendants in filling stations, who may be exposed to 50 mg/m^3 of methanol vapors for minutes at a time, many times a day.

Nevertheless, low-level exposures to methanol do cause small increases in blood and urine formate levels. Baumann and Angerer (1979) measured formate in 20 workers in a printing office who were exposed to an estimated methanol concentration of between 111 and 174 mg/m^3 of methanol throughout the work day. Over the course of a day, the blood level of formate rose an average of 4.7 mg/l (3.2 mg/l before the work

shift to 7.9 mg/l, when work ended), and urinary formate rose an average of 7.1 mg/l (13.1 to 20.2), both increases statistically significant. Every worker registered an increase for both parameters. A control group maintained relatively stable levels through the day (5.3 mg/l blood; 11.8 mg/l urine). Heinrich and Angerer (1982) performed a similar study in a chemical plant measuring methanol (blood and urine) and urinary formate in 20 workers exposed throughout the work day to 120 mg/m^3 methanol vapor, geometric mean (range of 48 to 300 mg/m^3). At day's end, blood and urine methanol in workers were 8.9 and 21.8 mg/l, respectively; a control group registered a mean blood value of less than 0.6 mg/l and a mean value in urine of 1.1 mg/l. Urinary formic acid was significantly higher in the workers (17.2 mg/l) than in the controls (12.7 mg/l).

Stegink et al (1981) assayed formate in the blood and urine of six adults who were given 200 mg/kg aspartame, equivalent to 20 mg/kg methanol. Blood methanol peaked at 26 mg/l, but no increase in blood formate was detectable over a pre-exposure value of 19.1 mg/l. Urinary formate increased in the 0-to-4 and the 4-to-8 hour post-ingestion periods to 2.9 and 2.4 times the pre-exposure levels (expressed as μg formate/mg creatinine); after the 8 hours post-ingestion period, urinary formate was at background levels. In addition, ophthalmologic examinations conducted 24 hours post-ingestion were all normal.
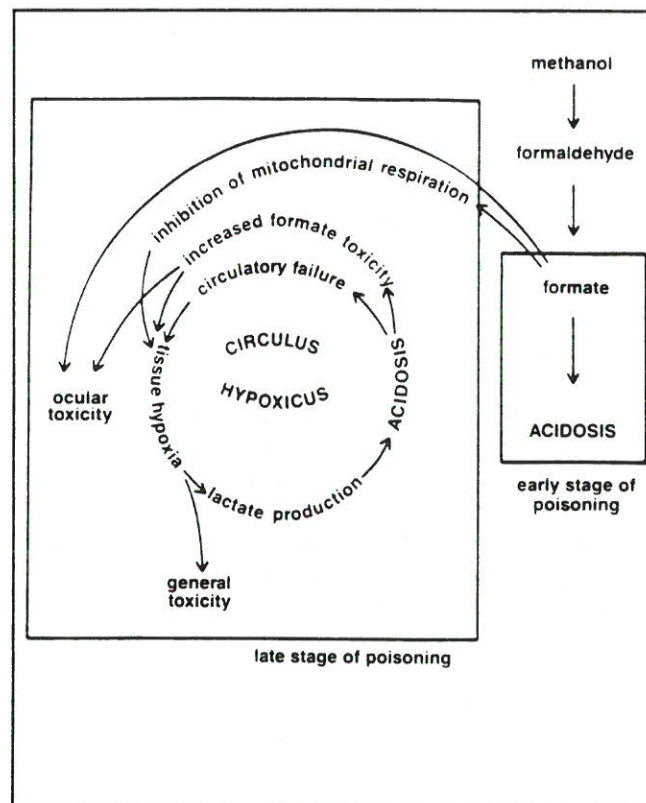
Figure 8: The circulus hypoxicus: a proposed mechanism for toxicity of methanol in humans

Source: Jacobsen and McMartin, 1986
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These studies present a wide variability in the background levels of blood formate. The source of the differences has not been identified, but may relate to the methods used to assay for blood formate, and, possibly, to dietary or other lifestyle or ethnic differences between the occupationally exposed populations in West Germany (Baumann and Angerer, 1979) and the adults studied in the United States (Stegink et al. 1981). These studies demonstrate that methanol exposures that do not challenge the metabolic limits of the folate pathway, nonetheless, do generate small amounts of formate in blood and urine. In the Baumann and Angerer (1979) study, workers were exposed to about 140 mg/m³ of methanol for 8 hours; assuming 20 m³/day ventilation and 100% absorption, the dose to a 70 kg person would be 13 mg/kg. According to Sedivec et al (1981) absorption might be as low as 60%, which would lower this estimate to perhaps 8 mg/kg. These investigators noted that blood formate increased from 3.2 mg/l to 7.9 mg/l over the course of the work day. In the aspartame study of Stegink et al (1981), the dose was higher (20 mg/kg), but the high background level of blood formate (19.1 mg/l pre-exposure) may have masked any subtle increases that the aspartame may have caused. Urinary formate rose in all three studies, which indicated that, following any low-level exposures to methanol (8 to 20 mg/kg), small quantities of formate probably are generated as well; but, unless the methanol is labeled in some distinct way, incremental formate may not be discriminated from background. In worst-case single exposure conditions of concern here (see above), it is doubtful whether or not an added body burden of 1 mg/kg or less of methanol will, once metabolized, impose measurably on background levels of formate.

Although incremental formate from ambient methanol exposures may not be measurable against background levels, sufficient data on methanol and formate kinetics are available to project estimates of formate accumulation following a single brief exposure to methanol vapor. Appendix VIII presents a two-compartment model in which the entire inhaled dose of methanol enters the metabolic pathway and is converted to formate with a rate constant, k_c, reflective of blood clearance, and formate is metabolized to CO_2 with a rate constant of k_f. An example in Appendix VIII models formate accumulation in an individual whose $T_{1/2}$ for methanol clearance is 3 hours, and whose $T_{1/2}$ for formate metabolism is 45 minutes (approximately the value measured by Malorny (1969b) in humans given formate orally). For such a person, an initial body burden of 1 mg methanol/kg that results from a worst-case exposure, generates, in the model, a peak formate level of 0.0082 mM (aqueous) at 2.0 hours post-exposure (see Figure VII-1). Because background formate is about 0.2 mM, the incremental contribution from methanol exposure is about 4%. For more realistic exposures, the model predicts considerably lower levels of methanol-derived formate. Finally, the example shows the sensitivity of formate accumulation to the efficiency of formate metabolism (Table VII-1). In short, decreased efficiency raises the peak formate concentration, as expected.

Although the model is only hypothetical, it is consistent with Stegink et al's (1981) inability to detect incremental blood formate levels following a 200 mg/kg dose of aspartame (equivalent to 20 mg/kg methanol). Because 1 mg/kg methanol projects for the subject in the above example to a peak of 0.0082 mM (aqueous) formate, 20 mg/kg would project proportionally to 0.16 mM or 7.4 mg/l; in terms of blood concentration, this peak value (assuming relatively even distribution of formate in body water) corrects to 6.1 mg/l. In Stegink et al's subjects, the pre-exposure blood formate level averaged 19.1 mg/l, roughly 3 times greater, and, in addition, displayed considerable variability around the mean. Thus it is not surprising that a relatively small and transient peak of blood formate, with its own inter-individual variability, was not detected in this study.

V. EVALUATION AND RECOMMENDATIONS

DISCUSSION

Exposure to methanol vapors will increase within the general public in the event methanol becomes a widely used vehicular fuel. The principal objectives of this report are (1) to evaluate whether or not health effects may be associated with such exposures, (2) to identify areas in which our knowledge is insufficient to draw conclusions, and (3) to recommend research that will help resolve current uncertainties. Based on EPA projections, the highest exposure levels will be encountered in personal garages during engine hot-soak and in filling stations during self-service operations, and, with increasing methanol penetration into the motor vehicle fleet, in public parking garages. These situations involve single exposures of less than 15 minutes to no more than about 200 mg/m³ methanol vapor. The methanol body burden that will result from a single worst-case exposure will be less than 1 mg/kg.

Methanol has been long recognized for its acute toxicity in humans, most frequently in association with ingestion of methanol, or wood alcohol-tainted beverages. The literature is replete with case histories of methanol poisoning, and its syndrome is well-characterized. Acute effects appear after a symptomless latent period of approximately a day, and consist of an acidosis with a superimposed ocular toxicity. Methanol intoxication may lead to blindness, and is potentially lethal. Susceptibility among individuals to methanol's acute effects is highly variable, but 300 mg/kg is considered to be at the low end of the dose range considered lethal. Variability may be associated with concurrent ingestion of ethanol, which slows the progression of methanol's toxicity, or with dietary status, which may play a role in susceptibility.

The recognition in the 1950s of the non-human primate as a model of human intoxication spurred research efforts that have produced a vastly improved understanding of methanol's toxic mechanisms. Methanol's toxicity is primarily attribu-

table to its metabolite, formate, which participates in the processes that lead to acidosis and visual disturbances. Methanol itself, unless taken in narcotic doses, is not considered the toxic principal in acute poisoning. In non-human primates, ocular effects are associated with blood formate concentrations in excess of 5 to 10 mM and require a day or more to express themselves morphologically (Martin-Amat et al. 1977; Martin-Amat et al. 1978). A methodical examination of potential effects in the visual system from lower levels of formate is lacking.

Two Russian human clinical studies suggest acute (i.e., during or immediately following exposure) neurobehavioral responses from brief exposures to methanol at concentrations below 5 mg/m^3 (Chao, 1959; Ubaydullayev, 1967). These responses were all sensory in nature, and involved olfaction, dark adaptation, and EEG patterns. The discussions in Section III and Appendix IV review these studies and present reasons for caution before accepting the authors' conclusions. The Russian findings must remain tentative, at best, pending replication.

The dose-effect relationships for methanol's chronic effects in humans are not well-described. On the basis of clinical case reports and a small number of epidemiologic studies, it appears that prolonged exposures to levels above the ACGIH TLV (260 mg/m^3) may produce effects akin to the symptoms described for acute toxicity, but less severe. These include headache and blurred vision. Acute and chronic effects may, to some extent, share common pathways of action.

The data base for animals exposed chronically to methanol is very limited, especially in non-human primates. In one chronic feeding study, infant stumptail macaques ingested up to 2.7 g aspartame/kg/day, i.e., 270 mg methanol/kg/day, for nine months with no physiologic or behavioral effects observed (Reynolds et al. 1984; Suomi, 1984). Histopathologic analyses were not included in these studies.

Cynomolgus monkeys exposed subchronically (6 hours per day, 5 days per week for 4 weeks) to methanol vapors at concentration levels of 650, 2,600, and 6,500 mg/m^3 displayed no signs of toxicity either during the exposure period or at post-exposure necropsy (Andrews et al. 1987). The latter included histologic analyses of several tissues, including conducting airways and lung, liver, kidney, and those tissues associated with the visual system. The New Energy Development Organization (NEDO) sponsored studies, in which cynomolgus monkeys were exposed for up to 30 months to 13, 130, and 1,300 mg/m^3. As discussed in Section III, the summary report issued by NEDO indicates potential chronic effects in the central nervous system at the lowest exposure level, but the details supplied in the report are insufficient to enable a critical review of the study, and further evaluation may be necessary.

Although non-primates do not serve as adequate models for the acute toxic syndrome, they may serve as subjects that can be used to explore for effects of methanol that are unrelated to its metabolism (i.e., direct effects). So far the data are limited, and do not reveal a firm indication that effects would result

from repeated human exposures under anticipated conditions. (Section III and Appendix IV)

A study of Sprague-Dawley rats (Appendix V) exposed for 4 weeks to methanol vapors (650, 2,600, and 6,500 mg/m^3) reports no exposure-related effects, except for increased discharges around the nose and eyes (Andrews et al. 1987). Cynomolgus monkeys exposed to the identical regimen showed no such effects (Andrews et al. 1987). In light of the latter observation, the biological significance of the increased discharges in rats is uncertain. In a behavioral teratology study, Infurna and Weiss (1986) report decrements in suckling and homing behaviors of rat pups (Long-Evans) born to mothers that had received methanol in their drinking water for three days during the third week of pregnancy (Appendix V). The maternal dose level was 2.5 g/kg/day, a level at least three orders of magnitude higher than the doses that will occur during expected worst-case exposures. Because of the absence of data on these behavioral endpoints at dose levels relevant to expected exposure conditions, the observations of this study remain interesting, but of limited value. Finally, as part of the large-scale research program sponsored by the New Energy Development Organization (NEDO, 1986), rats and mice were subjected to long-term exposures to methanol (13, 130, and 1,300 mg/m^3) to examine for toxicologic, as well as carcinogenic, effects (Section III). The summary report issued by NEDO indicates that rodents do not experience effects at 130 mg/m^3. However, as mentioned in the discussion of these studies in Section III, the results are not presented with sufficient detail to allow for critical review.

As indicated, single worst-case exposures will produce methanol body burdens of less than 1 mg/kg. Specifically, a 70 kg person breathing at a rate of 20 m^3/day exposed to 200 mg/m^3 of methanol for 15 minutes (i.e., hot-soak, worst case exposure), absorbing 100% of the inhaled vapor, receives a dose of 0.6 mg/kg (for 60% body water, this is an aqueous concentration of 0.03 mM). More realistically, a five-minute exposure to 50 mg/m^3 while refueling at the same ventilation, results in 0.05 mg/kg, or 0.003 mM. Background blood concentrations of methanol are about 0.75 mg/l, or, correcting for blood's water content (83%), about 0.9 mg/ml, or 0.03 mM aqueous concentration. Thus, for worst-case single exposures, the inhaled body burden of methanol will be equivalent to its pre-existing background level, but for more realistic exposures, it will be perhaps 10 times less. For comparison, aspartame in the diet is expected to produce additional methanol burdens that average between 0.3 and 1.1 mg methanol/kg/day (exclusive of carbonated beverages), and a single 12-ounce beverage will contribute about 20 mg, or 0.3 mg/kg, for a 70 kg person (Stegink, 1984).

The more important issue focuses on the generation of formate, and its potential toxicity. As indicated in the report, ambient exposures will not come near to challenging the folate pathway's formate metabolizing capacity, and, therefore, formate will not accumulate to frankly toxic levels. In one experiment (Stegink et al, 1981), in which adults were given

200 mg aspartame/kg orally (20 mg methanol/kg), increased formate was undetectable in blood against a background of 19.1 mg/l (0.4 mM). Urinary formate, however, increased about 2½-fold in the 8-hour post-ingestion period. In a second study (Baumann and Angerer, 1979), workers in a printing office who were exposed for the 8-hour work day to a mean 140 mg/m³ methanol vapor registered an increased blood formate of 4.7 mg/l (0.1 mM) at day's end against a pre-exposure level of 3.2 mg/l (the levels in unexposed controls was 5.3 mg/l); urinary formate increased as well. The relatively lower background in the latter study allowed the detection of the increased formate in blood. For such low-level methanol exposures, the increases of formate detectable in blood and urine reflect normally operating pathways of methanol metabolism.

As discussed in Section IV, total methanol exposure in these studies exceeds, by almost an order of magnitude, any single exposure expected from automotive or refueling vapors. The data from these studies combined with hypothetical modeling calculations suggest that single worst-case ambient exposures, i.e., 15 minutes exposure to 200 mg/m³ at twice resting ventilation, will, in a normal person, produce a temporary rise in blood formate of negligible magnitude (4%) compared to background levels. Exposures in traffic situations (Table 3), assuming 100% fleet penetration, will produce even less.

CONCLUSION

If methanol were to produce health effects in normal subjects at or near the exposure levels (time and concentration) of concern, such effects would likely not be attributable to the generation of formate. Susceptibility to methanol-derived formate remains unknown among people with dietary or metabolic deficiencies (e.g., conditions affecting the levels of liver folate or vitamin B_{12}), and remains unknown, as well, as a function of age. However, if formate is not active toxicologically and effects appear, an alternate mode of action (e.g., direct action of the alcohol) would need to be investigated.

The exposures of immediate concern will occur daily, but they will be brief and intermittent. If chronic exposures to equivalent concentrations do not produce health effects, one's confidence that anticipated conditions present no health hazard would certainly be enhanced. However, a firm conclusion regarding chronic exposure cannot yet be drawn. To this point, no human occupational studies have reported effects linked to chronic methanol exposures below the TLV (260 mg/m³). On the other hand, careful investigations of people exposed to lower levels have not been conducted, but would probably be very useful.

An analysis of the available peer-reviewed literature produces no evidence upon which to base a conclusion that exposure to low levels of methanol vapors will result in adverse health effects. This conclusion applies only to exposures that will occur as a result of methanol's normal use as a vehicular fuel, and not to exposures that may occur from ingesting methanol fuels, or from spillage.

RESEARCH OPPORTUNITIES AND ISSUES

The analysis presented in the report indicates that adverse effects are not expected as a result of exposure to methanol emissions from motor vehicles. Although several uncertainties have been identified regarding methanol's potential health effects, none are of a magnitude sufficient to justify investment of funds into a major program of research. Nevertheless, should methanol become widely implemented as a motor vehicle fuel, billions of gallons per year of this substance would be introduced into the fuel marketplace. With such large-scale usage, exposure to methanol emissions, though resulting in very low doses, is likely to be very widespread. Given this enormous scale, it is important to identify research issues and opportunities that, if addressed with focused research, would further reduce the level of uncertainty about health effects.

Much of the research to date has focused on the toxicity of methanol that follows large acute doses taken orally, and on refining clinical intervention strategies to treat that toxicity. Considerably less attention has been directed toward studying the effects that might occur following low-level chronic inhalation and the mechanisms that might be responsible. This report has reviewed the few studies concerned with low-level exposure, and has attempted to glean from the high-exposure studies the evidence that can be applied to the low-dose issues. Nonetheless, gaps in our knowledge about methanol's biological effects remain, and bridging them would provide the public with increased confidence in methanol technology.

At present, the worldwide level of effort in addressing methanol's health effects is quite small, and could be expanded to tackle specific research issues. The following discussion identifies six research opportunities that, if undertaken, would contribute valuable information on methanol's potential health effects. These research areas are not listed in priority order, and they do not represent a program that could be undertaken only with the limited resources of the Health Effects Institute as it is currently funded. Some of the problems, in a more refined form, may form the basis for future HEI work. Other problems will have to await increased interest from the relevant industrial, governmental, and scientific communities as the prospects for methanol fuels become clearer. In addition to evidence gathered from new research projects, important information may also be acquired from a re-analysis of Japan's New Energy Development Organization's study on non-human primates, and from unpublished data that may be available from studies conducted on the effects of aspartame that may be related to methanol.

The specific research opportunities are:

1. *Investigate dose-effect and time-course relationships between formate levels in blood and effects to the visual system.* According to current knowledge, the toxicity to the visual system in methanol poisoning is attributable to the generation and accumulation of circulating formate. However, the blood formate, in such cases,

achieves levels much higher than those that will arise from environmental exposures of the public to vehicular methanol vapors. Thus, research is suggested that will define the relationship between the concentration and duration of formate in the blood and the signs of toxicity to the visual system.

2. *Investigate local metabolism in the eye or optic nerve to determine if formaldehyde or formate is generated locally.* As indicated in the report, formaldehyde is not believed to be responsible for the known toxic effects of methanol. Methanol's metabolites, formaldehyde and formic acid, are generated mainly in the liver, and, at high enough doses, formate circulates to produce toxicity in the visual tract. One remaining uncertainty though, is whether metabolic systems are present in retinal or optic nerve tissues that also may generate toxic metabolites locally. The presence of such systems could have implications for evaluating the potential consequences of chronic, low-level exposure.

3. *Study potential susceptibility to low levels of methanol among people with dietary folate deficiency (or other suspect conditions) and within various age groups.* The report concludes, "if methanol produces health effects in normal subjects at or near the exposure levels of concern, such effects would likely not be attributable to the generation of formate." One of the major implications of this statement is that a similar definitive conclusion cannot be drawn for individuals who may be susceptible to methanol's effects. Furthermore, the Clean Air Act is written to ensure that potentially sensitive individuals remain protected. Thus, a potentially important research activity is to identify candidate determinants of susceptibility, and to develop strategies to study their role in modulating susceptibility within the population. To this end, the use of animal models may be appropriate.

4. *Conduct in-depth epidemiology study of workers exposed to methanol vapors.* As discussed in the report, studies have been conducted on various occupational cohorts exposed to methanol vapors that have measured blood and urine levels of methanol and formate. However, health-related parameters were not included in the design of these surveys. Methanol is used widely enough in various industries such that exposure information can be collected on selected cohorts and combined with data from health records, clinical examinations, and questionnaires to determine the extent to which chronic exposure to methanol affects workers, if at all.

5. *Conduct chronic animal experiments.* The most comprehensive animal research program undertaken to date was the one funded by the New Energy Development Organization. The report (Section III) indicates some of the difficulty understanding fully the findings that emerged from that program, particularly with reference to low-level chronic effects in non-human primates. A further difficulty arises with that study's design. Specifically, chronic exposures in the study occurred at methanol levels of 13, 130, and 1,300 mg/m^3. Even if a no-observed-effects-level of 130 mg/m^3 in this experiment (as the report suggests) is validated, uncertainty still persists about the possibility of effects occurring at levels somewhere between 130 and 1,300 mg/m^3, a range that includes exposure levels that may occur in several scenarios covered in Section II. Thus, a chronic study conducted with an appropriate animal model using well-chosen exposure levels, and engaging a full quality control and peer-review process, would constitute a valuable contribution.

6. *Conduct dose-effect studies that address behavioral teratology.* As discussed in the report (Appendix V), exposure of gravid rats to a single high dose of methanol produced behavioral decrements in pups tested in the early post-natal period. Unfortunately, data are lacking that describe the relationship between the level of maternal exposure to methanol and the behavioral performance of the offspring.