HEI HEALTH EFFECTS INSTITUTE

Reproductive and Offspring Developmental Effects Following Maternal Inhalation Exposure to Methanol in Nonhuman Primates

Part I: Methanol Disposition and Reproductive Toxicity in Adult Females
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Part II: Developmental Effects in Infants Exposed Prenatally to Methanol
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Includes the Commentary of the Institute's Health Review Committee

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HEI HEALTH EFFECTS INSTITUTE

The Health Effects Institute, established in 1980, is an independent and unbiased source of information on the health effects of motor vehicle emissions. HEI supports research on all major pollutants, including regulated pollutants (such as carbon monoxide, ozone, nitrogen dioxide, and particulate matter) and unregulated pollutants (such as diesel engine exhaust, methanol, and aldehydes). To date, HEI has supported more than 200 projects at institutions in North America and Europe and published over 100 research reports.

Typically, HEI receives half its funds from the U.S. Environmental Protection Agency and half from 28 manufacturers and marketers of motor vehicles and engines in the United States. Occasionally, funds from other public or private organizations either support special projects or provide resources for a portion of an HEI study. The study described in the Research Report was part of a larger methanol program that received some initial support from the American Petroleum Institute in addition to the funds provided by core sponsors. The Institute gratefully acknowledges substantial funding to Dr. Burbacher from the National Institute of Environmental Health Sciences for studies conducted on the second cohort of animals. Regardless of funding sources, HEI exercises complete autonomy in setting its research priorities and in reaching its conclusions.

An independent Board of Directors governs HEI. The Institute's Research and Review Committees serve complementary scientific purposes and draw distinguished scientists as members. The results of HEI-funded studies are made available as Research Reports, which contain both the Investigators' Report and the Health Review Committee's evaluation of the work's scientific quality and regulatory relevance.

HEI Statement

Synopsis of Research Report 89

Effects of Prenatal Exposure to Inhaled Methanol on Nonhuman Primates and Their Infant Offspring

INTRODUCTION

In an effort to improve air quality and decrease dependence on petroleum, the federal government, industry, and other groups have encouraged development of alternative fuels such as methanol to substitute for gasoline or diesel fuel. Methanol is also a candidate to provide the hydrogen for fuel cells, which are being developed for a variety of power sources (including motor vehicle engines). Before people are exposed to increased concentrations of methanol, the potential health effects of such exposures require study.

Methanol, a simple alcohol containing one carbon atom, occurs naturally in plants and animals and participates in human metabolism. People regularly consume low doses of methanol in fruits, vegetables, and fermented beverages as well as soft drinks and foods sweetened with aspartame (which breaks down to methanol in the gastrointestinal tract). Despite its ubiquitous presence, methanol can be highly toxic if sufficient quantities are consumed. Ingestion of methanol (usually in the form of wood alcohol or tainted alcoholic beverages) can result in metabolic acidosis, blindness, and even death. Although the body has the capacity to metabolize the low doses of methanol to which people are regularly exposed, it cannot handle high doses because too much methanol overwhelms the body's ability to remove a toxic metabolite (formate). When formate accumulates, methanol poisoning occurs. One factor that regulates the rate at which formate is removed is the liver level of a derivative of the vitamin folic acid. People who are deficient in folic acid (including 15% to 30% of pregnant women) may be particularly susceptible to the toxic effects of methanol.

If methanol were to be widely adopted as a fuel, environmental exposures would increase through ingestion of contaminated drinking water, inhalation of vapors from evaporative and other emissions, and dermal contact. Current concentrations of methanol in ambient air are very low, 1 to 30 parts per billion (ppb). If all motor vehicles in the United States were converted to 100% methanol fuel, methanol levels in ambient air are estimated to increase approximately 1,000-fold (to 1 to 10 ppm in cities) and in a worst-case situation could occasionally reach concentrations as high as 200 ppm in enclosed spaces (HEI 1987). Inhaling these concentrations of methanol for short periods of time is not predicted to affect formate production and thus should not present a health risk. However, little is known about the consequences of long-term inhalation of methanol vapors, especially in susceptible populations of pregnant women and developing fetuses. HEI, therefore, developed a research program to address this information gap.

APPROACH

Dr. Thomas Burbacher and colleagues of the University of Washington studied the effects of long-term exposure to methanol vapors on metabolism and reproduction in adult female monkeys (*Macaca fascicularis*) and developmental effects in their offspring, who were exposed prenatally to methanol.

The investigators exposed adult female monkeys (11 to 12 animals/group) to one of four concentrations of methanol vapors (0, 200, 600, and 1,800 ppm) for 2.5 hours a day, seven days a week during the following periods: (1) before breeding, (2) during breeding, and (3) during pregnancy. They collected blood from the adults at regular intervals to monitor methanol levels (which served as a marker of internal dose) and formate concentrations. They also conducted pharmacokinetic studies to determine whether methanol disposition (which includes absorption, distribution, metabolism, and excretion) was altered as a result of

This Statement, prepared by the Health Effects Institute and approved by its Board of Directors, is a summary of a research project sponsored by HEI. This study was conducted by Dr. Thomas Burbacher and colleagues of the University of Washington. The following Research Report contains both the detailed Investigators' Report (Parts I and II of Reproductive and Offspring Developmental Effects Following Maternal Inhalation Exposure to Methanol in Nonhuman Primates) as well as a Commentary on the study prepared by the Institute's Health Review Committee.

repeated methanol exposures and to assess pregnancy-related changes. Because high doses of methanol damage the central nervous system, the infants (8 to 9 animals/group) were examined at regular intervals during the first nine months of life to assess their growth and neurobehavioral development.

RESULTS

Exposure to methanol vapors did not affect the health of the adult monkeys prior to or during pregnancy. Single 2.5-hour exposures to methanol vapors caused short-term elevations in blood methanol concentrations of approximately 0- to 2-fold in the 200 ppm exposure group, 3- to 4-fold in the 600 ppm group, and 13- to 16-fold in the 1,800 ppm group. After long-term exposures, peak blood methanol concentrations declined slightly over the first month and remained constant thereafter. The concentrations of plasma formate (the toxic intermediate) remained at baseline levels during the entire course of the study in all exposure groups. Pregnancy had no effect on methanol disposition. Serum folate concentrations were not affected by pregnancy and methanol exposure.

Methanol exposure had no effect on most measures of reproductive performance, including menstrual cycles, conception rate, and live-birth delivery rate. However, all methanol-exposed animals had a decrease of about six to eight days in the duration of pregnancy compared to the control animals. It is not clear whether this decrease was related to methanol exposure as there was no dose response and no differences among offspring groups in body weight or other physical parameters. Prenatal exposure to methanol had no effect on infant growth and physical development for the first year of life. An unexplained wasting syndrome, characterized by growth retardation, malnutrition, and gastroenteritis, occurred after one year of age in two female offspring exposed in utero to 1,800 ppm methanol.

The investigators reported no systematic effects of prenatal methanol exposure on most of the measures used to test infant neurobehavioral development (neonatal behavior, early reflex responses, infant gross motor development, spatial memory, and social behavior). The investigators reported two possible methanol-related effects, one on visually directed reaching in male infants (a test of sensorimotor development), and one on novelty preference (a test of memory and cognitive function). Care must be taken in interpreting these results because a large number of neurobehavioral endpoints were analyzed and these results were based on a small number of subjects. Random fluctuations in the data may have appeared to be statistically significant. At the same time, however, both observations warrant further investigation as these central nervous system functions are complex perceptual processes that take time to develop and may be subject to latent neurotoxic effects.

CONCLUSIONS

This study adds substantially to our understanding of the effects of long-term exposure to inhaled methanol vapors. Because of the high quality of the study, the relevance of the animal model, the opportunities for dose-response analyses, and the availability of a marker of internal methanol dose, the results are appropriate for use in risk assessment. They can be readily used to predict the response of nutritionally competent people; they do not necessarily apply to women who are folate deficient.

The investigators' findings suggest that repeated inhalation exposure to concentrations of methanol vapors as high as 1,800 ppm would not result in accumulation of blood formate above baseline levels. With the exception of an unexplained shortening of gestation, methanol exposure had no effect on reproductive performance. The most significant result to emerge from this study was the wasting observed in two monkeys exposed in utero to 1,800 ppm methanol. Although this observation raises concern for prenatal exposures of this magnitude, pregnant women are unlikely to be exposed to such extremely high concentrations of methanol for prolonged periods of time.

Overall, the results provide no evidence of a robust effect of prenatal methanol exposure on the neurobehavioral development of nonhuman primate infants during the first nine months of life. However, improved understanding of methanol neurobehavioral toxicity will result from evaluation at later stages of development when more sophisticated tests of cognitive performance can be conducted and when latent effects may emerge. Such studies are now under way in the same monkeys at 4 to 5 years of age.

REFERENCE

Health Effects Institute. 1987. Automotive Methanol Vapors and Human Health: An Evaluation of Existing Scientific Information and Issues for Future Research. A Special Report of the Institute's Health Research Committee. Health Effects Institute, Cambridge, MA.

PREFACE

The use of methanol as an alternative fuel was proposed in the 1980s as a means to reduce petroleum imports, increase national security, and improve air quality (Gray and Alson 1989). Methanol appeared to be promising because it can easily and economically be produced from natural gas and a number of other feedstocks. Also, it has a high octane level and can reduce emission of hydrocarbons and other pollutants. More recently, methanol has emerged as a promising energy source for fuel cells, which are innovative electrochemical devices that convert fuel energy into hydrogen used to produce electric power for a variety of power sources (including motor vehicle engines). Although substituting methanol for gasoline and diesel fuels in motor vehicles would reduce the levels of some air pollutants, its expanded use raises concerns about the health implications of increased exposure of the general population to methanol vapors.

The prospect of introducing methanol as an alternative fuel prompted researchers and regulators to (1) resolve technical issues regarding fuel formulation and engine design, (2) characterize emissions from methanol-fueled vehicles and their effects on air quality, and (3) evaluate methanol's potential to cause health effects.

It is well known that methanol is toxic when ingested in sufficiently high amounts. In 1987, the Health Effects Institute (HEI) conducted an analysis of what emission-related health problems might emerge if methanol were to become more widely used as an automotive fuel. At that time, most information on the health risks of methanol exposure derived from clinical observations of humans who had accidentally or intentionally ingested methanol and from rodents exposed to very high concentrations of methanol. Human methanol poisoning is characterized by nausea, dizziness, metabolic acidosis, toxicity to the visual system (including blindness), and motor disturbances. If untreated, methanol poisoning can lead to coma and death (HEI 1987; Kavet and Nauss 1990; Kruse 1992; Marcus 1993; International Programme on Chemical Safety [IPCS] 1997). Visual toxicity is a hallmark of methanol intoxication. Clinical findings include visual disturbances, blood or edema in the optic disc, and an enlarged blind spot (Krause 1992). Autopsy reports have specified brain lesions, especially in the putamen and basal ganglia, in patients who died of a methanol overdose (Aquilonius et al. 1980; Koopmans et al. 1988; LeWitt and Martin 1988). The lethal methanol dose for humans is uncertain but appears to vary over a wide range (0.3 to 1 g/kg body weight) (IPCS 1997). Investigation of methanol toxicity in animals is difficult because normal rodents exposed to methanol do not display the metabolic acidosis and toxicity to the visual system that occur in humans (Roe 1982; Tephly and McMartin 1984; IPCS 1997).

The HEI Report (1987) found no evidence that short-term exposures to the concentrations of methanol vapors expected in ambient air as a result of methanol's use as a vehicular fuel would result in adverse health effects. The Report called for additional research, however, to reduce uncertainties regarding potential public health risks, especially the risks for susceptible subpopulations and the risks of prolonged low-level exposures. Because methanol was seriously being considered as a gasoline additive or replacement, HEI initiated a research program to address these issues. The study by Burbacher and colleagues, presented in this Research Report, was a key element in that program.

This Preface (1) provides background information on the sources of methanol exposures, (2) presents the regulatory context for the investigators' research, (3) briefly describes the known neurotoxic and developmental effects of methanol exposure, and (4) concludes with a description of the procedures that HEI used to develop its methanol research program and to review Dr. Burbacher's report.

METHANOL SOURCES AND USES

Methanol is a colorless, water-soluble liquid. Although methanol is a major chemical commodity, it also occurs naturally in humans, animals, and plants. In humans, methanol is derived both from the diet and from metabolic processes (Kavet and Nauss 1990; IPCS 1997). Dietary sources include fruits and vegetables, coffee, fruit juices, fermented beverages, and food containing the artificial sweetener aspartame, which hydrolyzes in the gut releasing 10% of its molecular weight as methanol (Stegink et al. 1981; IPCS 1997).

Methanol is widely used as a feedstock for chemical syntheses (for formaldehyde, acetic acid, and methyl tertiary-butyl ether [MTBE]) and as a solvent in a variety of consumer products (for example, paints and varnishes, antifreeze, windshield washers, cleansing solutions, and adhesives) (IPCS 1997; Malcolm Pirnie 1999). Methanol is also a component or byproduct in various commercial operations such as sewage treatment, fermentation, and the pulp and paper industry. In 1997, the U.S. Environmental Protection Agency (EPA) reported that methanol

ranked second for total release (221 million pounds annually) among chemicals listed on the agency's Toxic Release Inventory and first for air release (194 million pounds annually) (EPA 1999). However, it is the potential for expanded use of methanol as a vehicle fuel that has prompted research on the health effects of low-level exposures. Originally, proponents of methanol fuels envisioned their use as 100% (neat or straight) methanol, which is called M100. However, for technological, environmental, and safety reasons, most of today's methanol-based fuels are mixtures of 15% gasoline and 85% methanol, a blend known as M85.

Emissions testing of both light-duty and heavy-duty vehicles has shown that M85 significantly reduces emissions of carbon monoxide, hydrocarbons (including benzene and butadiene), and for heavy-duty vehicles, particulate matter (PM_{10}) (Gorse et al. 1992; Coburn et al. 1998). At the same time, methanol increases tailpipe emissions of methanol vapor as well as formaldehyde, which is toxic and has ozone-forming potential. Information on emissions from M100-fueled vehicles is more limited but generally shows the same trends (Auto/Oil Air Quality Improvement Research Program 1992). In addition to tailpipe emissions, inhalation of methanol vapor due to evaporative emissions from vehicles and fuel pumps, accidental ingestion from siphoning, and dermal exposure must be taken into account when considering possible human exposure to methanol as a consequence of its introduction into fuels.

REGULATORY AND POLICY ISSUES

In an effort to improve air quality and decrease dependence on petroleum, the U.S. government has encouraged the development of alternative fuels such as methanol by passing the Alternative Motor Fuels Act of 1988, the Clean Air Act Amendments of 1990, and the Energy Policy Act of 1992. As a result of these incentives, the petroleum industry has developed high-octane methanol fuels.

Under the provisions of the Clean Air Act, Congress authorized the EPA to establish national standards for air pollutants, set emission standards for motor vehicles, and regulate hazardous air pollutants. The original 1970 Act was amended in 1990, and those amendments deal with methanol in two ways—as a "hazardous air pollutant" (Section 112) and as a "clean alternative fuel" (Sections 241 and 242).

Section 112 of the Clean Air Act lists 189 hazardous air pollutants for which emission standards need to be set: methanol is one of those pollutants. In the 1990 amendments, Congress directed the EPA to review the list of hazardous air pollutants, to modify it as necessary, and to develop a national strategy to control their emissions from urban sources. Individuals and organizations have the right to petition the Agency to add to (or remove from) the list of hazardous air pollutants, substances for which the "emissions, ambient concentrations, bioaccumulation, or decomposition" are (or are not) reasonably anticipated to cause adverse health or environmental effects. The American Forest and Paper Association has submitted a petition requesting that methanol be delisted; this petition is now under consideration (Federal Register 1999).

The Clean Air Act Amendments of 1990 also established several programs for increasing the use of vehicles operated on "clean alternative fuels," which include fuels containing methanol. These amendments require the use of low-polluting fuels for buses in metropolitan areas if certain target levels are not met for ambient particles. In addition, the administrator of the EPA may extend the use of low-polluting fuels for other urban areas if "a significant benefit to public health could be expected to result from such an extension."

The Alternative Motor Fuels Act of 1988 is a federal statute that encourages widespread use of methanol, ethanol, and natural gas and the development of vehicles powered by these fuels. In 1992, Congress also passed the Comprehensive National Energy Policy Act to fulfill the low-polluting and clean-fuel mandates of the Clean Air Act Amendments of 1990 and to further stimulate alternative transportation fuels. The Act provides tax deductions for the purchase of alternative fuel vehicles and directs the U.S. Department of Energy to develop regulations to require the government and other organizations to acquire alternative-fuel vehicles. The final rule establishing the Alternative Fuel Transportation Program required "covered persons" to purchase increasing percentages of alternative fuel vehicles, beginning with 30% of the 1997 model-year light-duty vehicle acquisitions. To date, despite these efforts, methanol has not become an important component of the fuel supply in the United States except in its role as a feedstock for MTBE production.

Another important regulation would apply to methanol if it were proposed to be used widely as a fuel or fuel additive: Section 211 of the Clean Air Act mandates the EPA Administrator to require tests to determine the potential public health effects of fuels and fuel additives. Currently, no plans call for testing fuels containing methanol in conventional engines. Tailpipe emissions of many pollutants from fuel cell vehicles using methanol are much lower than those from conventional gasoline vehicles (Nowell 1999). Nevertheless, should methanol emerge as a fuel for

the generation of hydrogen in vehicles powered by fuel cells, this testing would likely need to commence in order to address the impact of evaporative emissions.

SCIENTIFIC BACKGROUND

The key to interpreting methanol toxicity is understanding its metabolism, including how species differ in the way they metabolize methanol and the impact of high-dose and low-dose exposures on these metabolic processes. Although methanol is metabolized through the same pathways in humans and animals, differences in the rate of formation of metabolic intermediates result in the marked variations in methanol-induced toxicity among species (Figure 1). After uptake (by either inhalation or ingestion) and distribution to body tissues, most methanol is metabolized in the liver to carbon dioxide and water; a small fraction is excreted directly through the lung and kidneys.

$$\underset{\text{methanol}}{CH_3OH} \xrightarrow{(1)} \underset{\text{formaldehyde}}{HCHO} \xrightarrow{(2)} \underset{\text{formate}}{HCOO^-} \xrightarrow{(3)} \underset{\text{water}}{H_2O} + \underset{\text{carbon dioxide}}{CO_2}$$

Figure 1. Methanol metabolism. (1) Reaction mediated by alcohol dehydrogenase in primates and by catalase in rodents. (2) Reaction mediated by formaldehyde dehydrogenase in both primates and rodents. (3) Reaction mediated by a number of enzymes that depend on liver tetrahydrofolate.

The first step in the metabolic sequence, the oxidation of methanol to formaldehyde, occurs at approximately the same rate in primates and rodents (Tephly and McMartin 1984). Formaldehyde is then rapidly oxidized to formate, the metabolite responsible for the toxic effects of methanol. The first two reactions are very fast, and formaldehyde has not been observed to accumulate in methanol-exposed animals or people. (However, some have suggested that given formaldehyde's high reactivity with cellular molecules, the failure to detect it does not preclude its possible involvement in methanol toxicity.) The key step in the metabolic pathway is the conversion of formate to carbon dioxide and water. This enzyme-mediated reaction requires the formation of a cofactor complex consisting of formate and tetrahydrofolate, which is derived from the vitamin folic acid (Makar and Tephly 1976). The concentration of tetrahydrofolate in the liver is a major determinant of the rate of formate removal (McMartin et al. 1977), and across species the rates of formate oxidation are directly related to liver levels of tetrahydrofolate. Removal of formate occurs about twice as fast in species that have high levels of liver tetrahydrofolate (rodents) as in those with lower levels (humans and other primates). In addition, levels of the enzyme responsible for oxidation of formate to carbon dioxide (10-formyl tetrahydrofolate dehydrogenase) are higher in rodents than primates (Johlin et al. 1987). The clinical effects of methanol depend on how much formate accumulates above the background level produced by normal metabolism (Tephly and McMartin 1984).

On the basis of limited emissions data, the concentrations of methanol vapors to which most people would be exposed if methanol were used as an alternative fuel are estimated to be too low to cause accumulation of formate and the onset of methanol toxicity. As reviewed by HEI (1987), ambient methanol concentrations resulting from vehicles operating on M100 are predicted to be 1 to 10 ppm in typical traffic situations but could be as high as 200 ppm in worst-case settings such as an enclosed garage with an engine idling or at hot ambient temperatures. Even these exposures are not expected to be high enough to cause an accumulation of formate above baseline levels in people with adequate folate levels (HEI 1987; Lee et al. 1992).

The above analysis suggests that inhaling low levels of methanol vapors should not present a health risk for most people because the body efficiently removes any excess formate. However, the clinical literature indicates that susceptibility to methanol toxicity varies widely (Kruse 1992; Marcus 1993). Because formate accumulation is a key factor in methanol toxicity, and its metabolism can be modified by liver folate concentrations or other factors that alter tetrahydrofolate regeneration, individuals who are folate-deficient may be at risk if exposed to methanol for prolonged periods of time. Pregnant and lactating women and patients with chronic alcoholism are among those with a high incidence of folate deficiency. It is well documented that folate deficiency during critical stages of pregnancy is associated with an increased risk of neural tube defects and that poor folate status may also be a risk factor for coronary disease (Selhub and Rosenberg 1990).* Animal studies have demonstrated that folate deficiency and treatments compromising the vitamin B_{12}–folate pathway decrease formate metabolism and exacerbate the toxic response to methanol (Eells 1992; Sahanashi et al. 1987). Thus, in some people who have mild folate deficiency, toxic symptoms could occur as a result of methanol exposures that are lower than those that cause toxicity in people who have adequate levels of folate.

* The Food and Drug Administration now requires U.S. Food manufacturers to fortify most enriched breads, flours, corn meal, pastries, rice and other grain products with folic acid to reduce the risk of neural tube birth defects in newborns.

The developing fetus may be particularly sensitive to the toxic effects of methanol exposure. The widely recognized teratogenic and neurotoxic effects of prenatal exposure to ethanol (which has similar physicochemical properties) raise concerns about the possible health effects of inhaled methanol vapors. Exposing the human fetus to high concentrations of ethanol (by maternal ingestion) can cause fetal alcohol syndrome, characterized by birth defects such as craniofacial malformations, growth retardation, and central nervous system disorders (Nulman et al. 1998; Mattson and Riley 1998). Consumption of moderate levels of ethanol can produce neurobehavioral deficits that are less severe but similar to those reported for fetal alcohol syndrome (Mattson and Riley 1998).

Evidence that methanol might be toxic for the developing fetus came initially from studies in laboratory rats indicating that fetal exposure to extremely high doses of methanol caused teratogenicity and neurotoxicity (Nelson et al. 1985; Infurna and Weiss 1986). Exposure of dams to 20,000 ppm methanol by inhalation for 7 hours daily throughout gestation increased the incidence of congenital malformations and urinary tract and cardiovascular defects in the offspring; lower exposure concentrations (10,000 ppm and 5,000 ppm) had no statistically significant effects (Nelson et al. 1985). Infurna and Weiss (1986) reported that early postnatal behavior (suckling behavior and time to locate nesting material) was delayed in rat pups after maternal ingestion of a high dose of methanol (2.5 g/kg/day) during gestational days 15 to 19.

More recent studies have not confirmed the teratogenicity of methanol in rats and point to differences in susceptibility between the mouse and the rat (Bolon et al. 1993; Rogers et al. 1993; Stanton et al. 1995; Weiss et al. 1996). Stanton and colleagues (1995) did not find significant changes in neurobehavioral and neurophysiological development in the offspring of rats exposed to 15,000 ppm methanol vapors (7 hours daily between gestational days 7 and 19). Weiss and coworkers (1996) also did not detect significant changes in a large number of behavioral endpoints (including suckling latency, motor activity, and cognitive function) in rats exposed to methanol (4,500 ppm/6 hours/day) in utero as well as after birth. In contrast, fetuses from pregnant mice exposed daily to very high concentrations of methanol vapors (5,000 to 15,000 ppm) from gestational days 6 to 15 showed an elevated incidence of exencephaly (failure of the skull to close over the brain) (Rogers et al. 1993). For this endpoint and under these exposure conditions, the parent compound methanol, rather than formate, may be responsible for the abnormality (Dorman et al. 1995). The difference in susceptibility of the two rodent species may be related to the higher blood methanol concentrations in mice than in rats exposed to the same concentrations of methanol vapors (Stanton et al. 1995; Pollack and Brouwer 1996).

Although toxicity resulting from single exposures to relatively high concentrations of methanol has been extensively studied, little information is recorded on the effects of prolonged, low-level exposures to methanol vapors. The most comprehensive study of the long-term effects of inhaled methanol vapors was conducted in rodents and monkeys by the New Energy Development Organization (NEDO) of Japan. The results have been presented in an abstract (Takeda and Katoh 1988) and in a report (NEDO 1987). Rats (F344/DuCrj) and mice (Crj:B6C3F$_1$) were exposed to methanol (0, 10, 100, or 1,000 ppm) for 12 or 24 months. No effects of exposure were reported for the two lower concentrations of methanol, but some increased body and organ weights were found in animals exposed to 1,000 ppm methanol. Pulmonary nodules increased in rats exposed to 1,000 ppm methanol; no methanol-related tumors were reported in mice. Two-generation reproductive studies in Crj:CD rats exposed continuously to methanol revealed no effects of methanol exposure on reproductive function (sexual cycle, days needed for insemination, insemination rate, pregnancy rate, litter size). The most notable finding was a decrease in brain weight in the offspring of rats exposed to methanol. Teratology studies were conducted on pregnant rats exposed to 0, 200, 1,000, or 5,000 ppm methanol for approximately 23 hours/day from day 7 to 17 of gestation. The highest exposure concentration (5,000 ppm) caused overt toxicity in the dams and fetal malformations in the first generation animals. Exposure concentrations of 1,000 ppm or less did not induce toxicity in the maternal animals, toxicity to the fetus, or effects on growth of the offspring.

NEDO also conducted long-term inhalation studies in monkeys (*Macaca fascicularis*) exposed to 10, 100, or 1,000 ppm methanol vapors for 21 hours/day for 7 months (2 animals/group), 1 year and 7 months (3 animals/group), or 2 years and 5 months (3 animals/group) (NEDO 1987). No changes were observed in physical growth or routine histologic parameters. The investigators reported degeneration of the basal ganglia of the cerebrum in animals exposed to 100 or 1,000 ppm methanol for 1 year and 7 months; this lesion was not found in animals exposed for 2 years and 5 months.

HEI METHANOL RESEARCH PROGRAM AND THE INVESTIGATORS' STUDY

As a result of concerns about the potential for health effects of methanol used as an alternative fuel, the HEI

Health Research Committee issued two Requests for Applications (RFAs) soliciting research proposals: RFA 87-1 "Behavioral and Neurotoxicological Effects of Methanol and Other Components of Automotive Emissions" and RFA 89-1 "Health Effects of Methanol Exposure: Metabolism and Pharmacokinetics; Fetal and Perinatal Neurotoxicity; Reproductive Toxicity."

RFA 89-1 requested proposals to study the metabolism of methanol following low-level exposures, especially in potentially susceptible populations, and methanol's effects on neurologic and reproductive function. Dr. Burbacher of the University of Washington submitted an application entitled "Primate Developmental Effects of Methanol," in which he proposed to evaluate the reproductive and developmental effects of methanol in nonhuman primates. After external peer review of all competing applications, the HEI Research Committee recommended Dr. Burbacher's proposal for one of the four studies funded under RFA 89-1. The Committee noted that the proposed study in macaque monkeys would provide critical information for risk assessments of methanol because of the similarities of humans and nonhuman primates in methanol metabolism, acute methanol-induced toxicity, placental structure, and neurobehavioral testing protocols. Because of the developmental and neurobehavioral effects reported in the offspring of pregnant rodents exposed to extremely high concentrations of methanol, it was important to confirm and extend these results using more sensitive endpoints, more suitable animal models, and more environmentally relevant methanol concentrations.

Dr. Burbacher's project was recognized as ambitious in that it involved exposure of adult female monkeys to methanol vapors by inhalation for a protracted period of time (before and during pregnancy) and behavioral testing of the offspring from birth to nine months of age. In order to achieve the desired sample size, the animals were divided into two cohorts, which were exposed and tested sequentially. Dr. Burbacher's study began in 1990 and lasted six years. HEI provided $2.6 million in funding for the project and the National Institute for Environmental Health Sciences provided $1 million to the investigator to support most of the costs associated with the second cohort of animals. The EPA supported maintenance of the infants after the 9-month testing period ended.

Three other studies funded under RFA 89-1 included a study on the uptake and disposition of methanol in rats and mice at different stages of gestation (Pollack and Brouwer 1996), a study on the effect of methanol on fetal development in rodents (Weiss et al. 1996), and a study on the uptake and disposition of methanol after a single exposure in nonhuman primates (Medinsky et al. 1997). This program provided comparative data on the metabolism and developmental effects of methanol across species.

All HEI-funded studies undergo an independent peer review of the methods, results, and data interpretation. Because of the complexity of his study, Dr. Burbacher reported the results in two parts. The investigators submitted a draft version of Part I, which addressed methanol disposition, adult toxicity, and reproductive toxicity, in March 1997. This report was evaluated by external peer reviewers and by members of the HEI Health Review Committee. Burbacher and colleagues submitted a revised draft of Part I in February 1998. In January 1998, they submitted a draft of Part II, which contained the developmental neurotoxicity results for the infant monkeys exposed to methanol in utero. HEI formed a Review Panel consisting of Review Committee members and consultants with expertise in pharmacokinetics, toxicology, and neurobehavioral toxicity to assist in the review of these reports. In March 1998 the panel met with the investigators and in executive session to discuss Parts I and II. Dr. Burbacher also presented his preliminary findings at the HEI Annual Conference in April 1998. On the basis of these discussions, the panel requested further revisions to Part I and additional analyses for Part II. The investigators submitted the revised final drafts, which form the basis of this Research Report, in July 1998 (Part I) and September 1998 (Part II). The panel reviewed these drafts and subsequent revisions that were made during the editorial process.

The Review Committee discussed the revised reports and the panel's evaluation and recommended Dr. Burbacher's reports for publication. The Committee, with the assistance of the panel and staff, then prepared its Commentary, which is included in this Research Report. The Review Committee's Commentary is intended to aid HEI's sponsors and the public by highlighting the strengths and limitations of the study and by placing the investigators' findings into scientific and regulatory perspective.

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HEI REVIEW COMMITTEE

The Review Committee gratefully acknowledges the cooperation of the investigators during the review process and the work of the members of the Review Panel who assisted the Committee in evaluating the Investigators' Report and developing its Commentary.

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