

HEI

COMMUNICATIONS

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Preface

The goal of this report produced by the Health Effects Institute (HEI) is to provide information to decisionmakers on research that is potentially capable of narrowing uncertainties related to the health effects of specific air toxics. This report is based on the Mobile Air Toxics Workshop organized by HEI in Monterey, CA, on December 4-6, 1992.

At the workshop, approximately 50 scientific experts divided into five working groups on benzene, 1,3-butadiene, formaldehyde and acetaldehyde, and polycyclic organic matter, all of which are specified as mobile source toxic air pollutants in the Clean Air Act and methanol, a potentially important alternative fuel. Table 1 lists Working Group members, chairs, rapporteurs, and HEI staff participants. Rapporteurs were chosen from members of the HEI Research and Review Committees. Certain Working Group members also were assigned the role of assessing cross-cutting issues (see Table 1). In addition to the Working Group participants, there were 32 observers, primarily from government and industry, who participated in the discussions but not in the writing of the document. The initial and final drafts were sent for comment to all workshop attendees and to a group of external reviewers listed in Table 2. Working Group chairs worked with rapporteurs and HEI staff in responding to reviewers' comments.

A second meeting was held on January 13-14, 1993 at the offices of HEI in Cambridge, MA. The meeting was attended by Working Group chairs, rapporteurs, and those responsible for cross-cutting issues; they focused on responding to comments and identifying gaps.

Why focus on mobile air toxics and on these specific compounds at this time? Section 202(l) of the 1990 Amendments to the Clean Air Act creates a new priority for the study and regulation of mobile air toxics. Section 202(l)(1) specifies that within 18 months after the date of enactment of the amendments (October, 1990), the EPA "Administrator shall complete a study of toxic air pollutants... which are unregulated under the Act" to evaluate the need for and feasibility of controlling such pollutants. The Act specifies that "the study shall focus on those categories of emissions which pose the greatest risk to human health or about which significant uncertainties

remain." Section 202(l)(2) requires the Administrator to promulgate (and from time to time revise) regulations to control hazardous air pollutants from motor vehicles and motor vehicle fuels based on the study within 54 months after enactment of the amended law (May 1995).

These new 202(l) regulations are important in several respects. They add specificity and time deadlines to the development of data on the regulation of mobile source unregulated pollutants. Therefore, they add definition to the general responsibility of HEI, which is to provide data to both the EPA and the motor vehicle industry on unregulated pollutants that may pose an unreasonable risk to human health, welfare, or safety. This mandate falls under Sections 202(a)(4) and 206(a)(3) of the Clean Air Act. Furthermore, these regulations are unique in Title II of the Clean Air Act in specifying that the Agency may regulate either motor vehicles or motor vehicle fuels in order to achieve any needed reductions.

The EPA and members of the motor vehicle industry urged HEI to conduct this workshop and to prepare this report, in part to complement the Agency's study required under Section 202(l)(1), and to help focus research on certain unregulated mobile source emissions, especially those compounds that are specified as mobile source toxic air pollutants in Section 211(k)(10). The EPA study, under way when HEI's workshop was being planned, was issued in April 1993¹. That study clearly brought out the uncertainties remaining in evaluating the effects of mobile air toxics, particularly at the low levels to which human beings may be exposed.

The choice to assess these particular compounds at the workshop and in this report, then, was based upon specific mention of them in the Clean Air Act as well as their potential for adverse health consequences. Working Groups were instructed to keep in mind the shorter term needs that the U.S. EPA faces with legislative deadlines (and similar needs of the California Air Resources Board). The pertinent laws, including the Clean Air Act of 1990, were presented and discussed at the workshop. This information was a useful guide to deliberations of the Working

¹ U.S. Environmental Protection Agency. 1993. Motor Vehicle-Related Air Toxics Study. EPA 420-R-93-005. Office of Mobile Sources, U.S. Environmental Protection Agency, Ann Arbor, MI.

Groups. Discussants pointed out that the control or prevention of health effects of existing air toxics will continue to be contentious and that technological advances will present new challenges.

Because the rationale for planning the Workshop was gathering data toward the eventual control of potentially hazardous mobile source emissions, the focus was on research needs related to understanding the effects of these agents at relevant concentrations in the air. For example, studies of workers exposed to relatively high concentrations of a mobile air toxic might be recommended if the findings could be used to help estimate effects at the lower concentrations expected to be present in the general community, but not if the findings were solely pertinent to workers' health.

The focus of this workshop was to identify and prioritize research needs related to decreasing uncertainties in risk assessments for these compounds. The Working Group members were specifically told not to resolve differences in opinion related to the existing scientific data base. Accordingly, this document should not be considered to be an authoritative interpretation of existing scientific information about these pollutants; nor does it necessarily represent the views of any of the sponsoring organizations or HEI. Rather, the brief review of current understanding, including differences of scientific opinion, is provided as a background to explain the rationale for the recommended research. Although there is some general consistency of format across chapters, each Working Group was faced with different challenges, which necessitated different structural approaches.

The decision to have a chapter on cross-cutting issues reflected the realization that there are a number of generic issues pertinent to more than one of the mobile source air toxics. Our goal is two-fold. We hope that the presentation of cross-cutting issues will give the reader a better understanding of certain of the basic conceptual problems underlying the assessment of risks due to mobile source air toxics. Furthermore, because many of these issues are pertinent to more than one agent, they deserve a higher research priority than do some of the research needs specific to a single pollutant.

The research recommendations in this document are aimed at all organizations funding research on the health effects of mobile source air

toxics and are not intended to be restricted solely to the planning needs of HEI. The Health Effects Institute's relatively minimal budget has always put it in the position of being a small piece of the overall research program in automotive air pollutants, but one with a specific focus on enhancing the output of the scientific community in responding to the need for credible decision-making. We hope that the document will serve as a basis for research planning by the many governmental and industrial organizations that need to make appropriate decisions about the health risks of mobile source air toxics. We note that the economic impact of regulatory changes that respond to the concern about the health effects of mobile air toxics can be enormous: consider that an increase in gasoline price of one cent per gallon is equivalent to about \$1 billion yearly. The past experience of only a relatively trivial investment in research aimed at reducing uncertainties concerning mobile source air toxics must not be repeated if we are to deal effectively with this potential health threat from exposure to mobile air toxics.

Research can be described as going up alleys to see if they are blind. The present document focuses on those alleys that seem to provide the best likelihood of relatively quickly providing an opening into the light of new information of relevance to decisionmakers. As with any research effort, it is difficult to make predictions about where the alley goes and how long it will take to get to its end. For those of us with experience in research planning in the environmental health sciences, the one certainty is that in the next few years there will be a major scientific advance of crucial importance to understanding the potential health impact of air toxics that is not now predicted by anyone in this workshop. The implication of this one certainty is that the research planning process and the scientific community must maintain flexibility so as to be able to respond to the inevitable rapid developments in the field of biological sciences.

I would like to thank Jack Moore and Roger McClellan, Vice-Chairs of the Workshop, for their valuable contributions in planning the program and their active involvement in the workshop activities.

Bernard D. Goldstein
Workshop Chair

Table 1. Working Group Members

Working Group Members	Benzene	Butadiene	Formaldehyde	Methanol	Polycyclic Organic Matter
Chairs	<i>George Lucier</i> NIEHS ^a	<i>Roy Albert</i> U. Cincinnati	<i>Rory Conolly</i> CIIT	<i>Bernard Weiss</i> U. Rochester	<i>Joe Mauderly</i> ITRI
Rapporteurs	<i>Arthur Upton</i> NYU Medical School, HEI Review Committee	<i>Stephen Hecht</i> American Health Foundation, HEI Research Committee	<i>Meryl Karol</i> U. Pittsburgh, HEI Research Committee	<i>Gareth Green</i> Harvard School of Public Health, HEI Review Committee	<i>Herbert Rosenkranz</i> U. Pittsburgh, HEI Review Committee
Cross-cutting experts	High-to-low dose extrapolation <i>Daniel Krewski</i> Health & Welfare, Canada	Epidemiology <i>Genevieve Matanoski</i> Johns Hopkins U.	Dosimetry <i>Henry Heck</i> CIIT	Developmental, neurotoxic and reproductive effects <i>Thomas Burbacher</i> U. Washington Exposure assessment <i>Paul Lioy</i> EOHSI	Molecular biology <i>Seymour Garte</i> NYU Medical School, HEI Research Committee
Others	<i>Jerry Blancato</i> U.S. EPA	<i>John Acquavella</i> Monsanto Co.	<i>James Ball</i> Ford Motor Co.	<i>Janis Eells</i> Medical College of Wisconsin	<i>John Froines</i> UCLA
	<i>Robert Drew</i> American Petroleum Institute	<i>George Alexeeff</i> California EPA	<i>Joan Daisey</i> Lawrence Berkeley Lab.	<i>Eun Woo Lee</i> General Motors Corp.	<i>Uwe Heinrich</i> Fraunhofer Institute
	<i>Carl Mackerer</i> Mobil Oil Corp.	<i>Michael Bird</i> Exxon Biomedical Sciences, Inc.	<i>Carol Henry</i> California EPA	<i>Michele Medinsky</i> CIIT	<i>Edmond Lavoie</i> Rutgers U.
	<i>Christopher Portier</i> NIEHS	<i>James Bond</i> CIIT	<i>Mary Henry</i> U.S. EPA	<i>John Rogers</i> U.S. EPA	<i>Irving Salmeen</i> Ford Motor Co.
	<i>Robert Snyder</i> EOHSI	<i>Rogene Henderson</i> ITRI	<i>Judith MacGregor</i> Toxicology Consulting Services		<i>Joellen Lewtas</i> U.S. EPA
	<i>Jaroslav Vostal</i> General Motors Corp.	<i>Richard Irons</i> U. Colorado	<i>Andrew Salmon</i> California EPA		
	<i>Greg Wilkinson</i> U. Texas	<i>Ronald Melnick</i> NIEHS <i>Lorenz Rhomberg</i> U.S. EPA			
HEI staff	<i>Martha Richmond</i>	<i>Jean Murphy</i> <i>Kathleen Nauss</i>	<i>Brenda Barry</i> <i>Jane Warren</i>	<i>Maria Costantini</i>	<i>Debra Kaden</i>

^a NIEHS = National Institute of Environmental Health Sciences; CIIT = Chemical Industry Institute of Toxicology; ITRI = Inhalation Toxicology Research Institute; NYU = New York University; EOHSI = Environmental and Occupational Health Sciences Institute; UCLA = University of California at Los Angeles.

Table 2. Reviewers of Draft Chapters^a

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Massachusetts Institute of Technology

^a HEI acknowledges with appreciation the thoughtful comments of these reviewers. Their suggestions and criticisms led to many modifications that improved this report. We point out, though, that the reviewers do not necessarily agree with all of the contents of the report and are, of course, not responsible for them.

Research Priorities to Reduce Uncertainties in Risk Assessment for Aldehydes

INTRODUCTION

Aldehydes are a group of compounds produced by incomplete combustion of fossil fuels and other organic materials. Aldehydes are also synthesized by the chemical industry for use in a wide variety of products. The aldehydes formaldehyde, acetaldehyde, and acrolein are constituents of the emissions produced by motor vehicles during combustion of gasoline and diesel fuels (Marnett 1988). Formaldehyde and acrolein are also used in the manufacture of many home products, chemicals, plastics, and textiles (Feinman 1988). Because of the diverse applications for aldehydes, human exposures can occur in numerous occupational settings as well as in the home and out-of-doors. Outdoor exposures to formaldehyde and acetaldehyde may increase in the future due to a projected increase in the use of methanol and ethanol as alternative fuels and in fuel blends. This projected increase is based on the fact that combustion of alcohol fuels by motor vehicles produces more aldehydes than combustion of conventional gasoline and diesel fuels.

Effects from acute exposures to aldehydes can range from eye and throat irritation in humans and animals to genetic damage in isolated cells exposed under culture conditions. The severity of these effects depends on the toxicity of the particular aldehyde as well as the exposure concentration and duration of exposure (Leikauf 1992). Based in part on the clear link between chronic exposures to inhaled formaldehyde and cancer development in animals, and on suggestive epidemiological evidence in humans, formaldehyde has been categorized as a group B1 probable human carcinogen by the U.S. Environmental Protection Agency (EPA) (1987a, 1991). Because fewer data are available for acetaldehyde, the EPA (U.S. Environmental Protection Agency 1987b) has classified this compound as a group B2 probable

human carcinogen.

In view of the known and potential health effects of formaldehyde and acetaldehyde, Section 211 of the Clean Air Act Amendments of 1990 (U.S. Congress 1991), which deals with regulation of fuels, defines these compounds as toxic air pollutants. In addition, Section 202 of the Act specifies further study regarding the need for and feasibility of controlling formaldehyde emissions from mobile sources. Because of these specific regulations for formaldehyde and acetaldehyde, the Aldehydes Working Group discussed both of these aldehydes. The Working Group acknowledged that much more scientific literature is available for formaldehyde than for acetaldehyde and that the research issues underlying formaldehyde risk assessment are correspondingly better focused than they are for acetaldehyde. As a result, the Working Group concentrated its efforts on identifying research priorities that would reduce the uncertainties in risk assessment for formaldehyde and allocated less attention to acetaldehyde. The Working Group members also recognized that other aldehydes, including acrolein and propionaldehyde, were included in the list of 189 hazardous air pollutants enumerated in Section 112 of the Clean Air Act. Although beyond the scope of this Working Group, issues related to research needs and risk assessments for these aldehydes and acetaldehyde are worthy of future evaluation.

EXPOSURE ASSESSMENT

Exposure is defined as contact at an external boundary between a human and an agent, for a specified period of time, at a specified concentration, and in a specified medium of exposure. Exposures can occur when contaminants enter the body through various routes, such as by inhalation, ingestion, and dermal absorption, and via various media, such as air, food, water, and clothing. For risk management, for epidemiology studies, and for research planning in general, it is important to understand the percentage that each of these various routes and exposure media contributes to the total human exposure. In the case of aldehydes, there is evidence that exposures occur through a variety of routes and media, but the relative contributions of each have not yet been determined.

Aldehydes Working Group: Rory B. Conolly (Chair), Meryl H. Karol (Rapporteur), James C. Ball, Brenda E. Barry, Joan M. Daisy, Henry d'A. Heck, Carol J. Henry, Mary Henry, Judith MacGregor, Andrew Salmon, and Jane Warren.

Indoor environments must be recognized as a significant source of human exposures to aldehydes. On average, people in the United States spend approximately 70% of their time indoors at home and 20% indoors at work. The contribution of these indoor scenarios to total human exposure levels is particularly relevant to formaldehyde because indoor formaldehyde levels often exceed outdoor levels. These higher indoor levels are due to a variety of sources, including pressed wood products, furnishings, textiles, clothing, environmental tobacco smoke, kerosene heaters, gas burners, and building materials such as urea formaldehyde foam insulation. Although outdoor aldehyde levels presumably affect indoor concentrations through seepage indoors, no experimental data exist for the fraction of indoor levels that originate from outdoor sources.

In outdoor air, aldehydes originate from direct emissions of both mobile and stationary sources and are also produced in the atmosphere through photochemical reactions with precursors. As a result, individuals may receive significant outdoor exposures to aldehydes while commuting to work and exercising.

What Is Known About Exposures to Aldehydes?

Estimates have been made for atmospheric levels of some common pollutants present in mobile source emissions (Graedel 1988). Formaldehyde and acetaldehyde are usually the most abundant aldehydes in these emissions, and as a result, far more is known about their potential health effects. However, little information is available regarding exposures of animals or humans to other aldehydes such as propionaldehyde, which is listed as a hazardous air pollutant in the 1990 Clean Air Act Amendments.

The distribution of population exposures to formaldehyde and acetaldehyde—that is, the ranges of the exposure levels and the numbers of individuals exposed to these various levels—has not been documented as well as it has been for radon and benzene. However, some information about ranges of indoor and outdoor air concentrations of aldehydes and about sources is available. Some of these data are presented in Table 1.

Several things are apparent from this table. First, aldehyde concentrations in indoor and outdoor exposure scenarios range from a few ppb

to several hundred ppb. These concentrations are usually well below the level of one ppm or higher that is often used in experiments with laboratory animals. However, some of the higher formaldehyde levels that have been recorded indoors can produce irritant effects in humans. The second point is that, compared to formaldehyde, fewer measurements have been made for indoor acetaldehyde levels. Exposures to these two aldehydes typically occur in conjunction with many other airborne pollutants that are also present at ppb levels. The potential additive and synergistic effects of exposures to mixtures of aldehydes as well as aldehydes combined with other air pollutants are not well understood.

Increased use of oxygenated fuels and shifts to methanol-fueled vehicles must be considered as potential sources of increased outdoor formaldehyde and acetaldehyde levels. Data from post-1989 vehicles fitted with catalytic converters and fueled with a blend of 85% methanol and 15% gasoline indicate that they emit three to six times more formaldehyde per mile than conventionally fueled vehicles (National Research Council 1992). Vehicles fueled with pure methanol emitted slightly more formaldehyde per mile than vehicles using blended fuels. With both fuels, the cold-start phase of engine operation contributed a substantial percentage of the total emission amounts. Despite these increased formaldehyde emissions per mile, significant increases in outdoor levels of formaldehyde are not anticipated because most outdoor formaldehyde is photochemically formed. Along a similar line, photochemical reactions of methanol vapors in the atmosphere are not expected to increase outdoor formaldehyde levels because methanol is less reactive than gasoline. However, emissions of formaldehyde into the closed spaces of garages and tunnels conceivably could lead to levels high enough to cause irritant effects.

Addition of the fuel additive methyl t-butyl ether (MTBE) to gasoline has yielded a more oxygenated fuel that produces less carbon monoxide during combustion. However, based on estimates by the EPA (U.S. Environmental Protection Agency 1992), the presence of this additive also increases the formaldehyde levels in engine emissions compared with previous fuels. A significant increase in total outdoor levels of may have a more substantial influence on these ambient levels. The impact of this additive on

Table 1. Concentrations of Formaldehyde and Acetaldehyde in Indoor and Outdoor Air^a

Location	Type	Formaldehyde (ppb)	Acetaldehyde (ppb)	Reference
Southern California				
1977-1989	Outdoor	0.4-90	-	Grosjean 1991
1988-1989	Outdoor	~1-29	~1-13	Grosjean 1991
Sao Paulo, Brazil ^b	Outdoor	2-19	0.9-19	Grosjean et al. 1990
8 U.S. homes	Indoor	5-19	2-21	Lewis and Zweidinger
8 U.S. homes	Outdoor	3-7	1-4	Lewis and Zweidinger
Homes and offices	Indoor	5-800	-	Ota and Mulberg 1990
>500 Mobile homes	Indoor	10-460	-	Liu et al. 1991
Fabric stores	Indoor	30-280	-	McGuire et al. 1992
Motor vehicles	In Car	12	8	Shikiya et al. 1989
Parking garage	Indoor	34	-	Wilson et al. 1991
Service station	Outdoor	4	-	Wilson et al. 1991

^a The data in this table are intended to be an illustrative, rather than a comprehensive representation of exposure

^b Ethanol used in motor vehicle fuel.

formaldehyde is not expected, but photochemical reactions of unburned MTBE in the atmosphere outdoor formaldehyde levels should be evaluated carefully with regard to current formaldehyde levels and exposure scenarios.

Although acetaldehyde and formaldehyde are recognized as normal food constituents, the contribution of their ingestion to total human exposure has not been determined. Acetaldehyde is also a major metabolite of ethanol. Consequently, ethanol ingestion may be a more significant source of acetaldehyde exposure and any subsequent increased cancer risk than food or air, but this has not been fully evaluated.

There is also reason to suspect that dermal exposures to formaldehyde may be significant. Formaldehyde is used in the manufacture of many clothing fabrics; thus, this compound may be absorbed through the skin when relatively new garments are worn. Some cosmetics, toiletries, and household products also contain formaldehyde that can be absorbed through the skin during product use. The extent to which these products and routes

of exposure contribute to total human formaldehyde burden has not yet been determined adequately.

Much research has focused on the risk that formaldehyde and acetaldehyde present as cancer-causing agents. Current measurements suggest that a significant percentage of the population may be exposed to the aldehydes at levels that produce irritant effects. We do not currently know the distribution of population exposures.

Research Recommendations.

Despite our current knowledge about the sources and the ranges of indoor and outdoor air concentrations for formaldehyde and acetaldehyde, several significant gaps exist in our knowledge that are important for risk assessment and risk management of these compounds. These are listed below.

- *Determination of total human exposures to formaldehyde and acetaldehyde by all routes and the relative contribution of each route.*

- *Development of methods for measuring short-term peak exposures to aldehydes in typical non-occupational environments because it is likely that some of these levels will be high enough to cause irritant effects.*
- *Evaluation of the distribution of population exposures to formaldehyde and acetaldehyde.*
- *Determination of the fraction of outdoor formaldehyde and acetaldehyde that penetrates into buildings. The contribution of outdoor sources to indoor exposures is important for appropriate risk evaluation. Because some significant fraction is also lost via sorption on surfaces, further investigation of this effect is necessary.*

CANCER RISK ASSESSMENT

Epidemiology

Ideally, human health risk estimates for formaldehyde and acetaldehyde should be based on human data. This approach avoids the need for animal experimentation and the complicated, and sometimes uncertain, process of extrapolating animal data to human exposures. Estimates of the human cancer potency of formaldehyde based solely on data from rat studies are very uncertain due to the steep dose-response curve and an absence of information at the low doses actually experienced by humans. Current calculations for humans involve both high-dose to low-dose extrapolations and interspecies comparisons. Another uncertainty in these extrapolations is that formaldehyde exposure may produce cancer in humans at sites other than the nasal cavity, such as the nasopharynx. Although the biological support is not strong, some epidemiologic evidence also indicates that formaldehyde exposure is associated with an increased incidence of leukemia and brain cancer in exposed humans (U. S. Environmental Protection Agency 1991).

Although little epidemiologic information is available for acetaldehyde, formaldehyde has been well studied (California Environmental Protection Agency 1991, U.S. Environmental Protection Agency 1991). Blair and colleagues (1990) performed the only metaanalysis to date using an epidemiology data base obtained from groups of individuals that had been occupationally exposed to

formaldehyde. Their findings provided limited evidence that formaldehyde was a human carcinogen. On the basis of this limited evidence in humans and the unequivocal evidence that inhaled formaldehyde produced cancer in rats, the International Agency for Research on Cancer (IARC), the U.S. EPA, and the California EPA characterized inhaled formaldehyde as a probable human carcinogen. However, the data were found to be insufficient to provide a quantitative assessment of cancer potency based on human exposure and tumor incidence data.

It would be useful to establish a quantitative exposure-response relationship for humans that have been exposed to formaldehyde and to derive a cancer potency value. It certainly should be possible to derive several important pieces of information from the human data base, in particular, the plausible constraints on the potency estimates extrapolated from the animal studies. To derive such information, further examination of the existing epidemiology data, including analysis of the original exposure and incidence data, is recommended. This research approach would be an expeditious way to improve the formaldehyde potency estimate and resulting risk predictions. Generically, the interspecies extrapolation is particularly problematic for formaldehyde as extensively reviewed in other sections of this chapter and by the regulatory agencies (California Environmental Protection Agency 1991, U.S. Environmental Protection Agency 1991). Determining some bounds on the human potency is critical because of the diverse patterns of low level exposures to formaldehyde.

Research Recommendations.

- *A new metaanalysis of the existing epidemiology data for formaldehyde, including analysis of the original exposure and incidence data, is recommended. Ideally, such an analysis would establish a quantitative exposure-response relationship in humans that could be used to derive a cancer potency value. To provide the best exposure-response estimate, the metaanalysis should be restricted to well-designed and well-conducted studies, particularly with regard to exposure assessment. It would be instructive to compare the potency values obtained from these analyses with those for rats.*

- *A less ambitious, though possibly more realistic goal for this study would be to identify plausible constraints in the potency estimates that are extrapolated from the animal studies to humans.*

Regional Tissue Dosimetry

Chemical carcinogenesis initially depends on two broad groups of processes, pharmacokinetics and pharmacodynamics. Absorption, metabolism, distribution to tissues, and excretion can be grouped under pharmacokinetics. Pharmacodynamics refers to the processes by which chemicals produce lesions in the DNA or, in the case of nongenotoxic carcinogens, bind to receptors and subsequently result in cell transformation and tumor growth. Both pharmacokinetic and pharmacodynamic processes vary among species. There is little reason to expect that the interspecies variation in pharmacodynamics should necessarily parallel those in pharmacokinetics or vice versa.

Because of the fundamental division between these two groups of processes, it is important to address them separately. Substantial pharmacokinetic data are available for various laboratory animal species, but similar data for humans are more limited. Combined with appropriate mathematical models, it may be possible to use these data to analyze both the dose dependence and interspecies variation of pharmacokinetics for tissue doses of reactive metabolites. Although such pharmacokinetic analyses are neither simple nor complete, they represent a much greater depth of understanding than is currently available for pharmacodynamic processes.

When formaldehyde and acetaldehyde are inhaled, their primary target is the lining of the respiratory tract. However, our concern focuses not only on the total amount of each compound that enters the respiratory tract, but also on the role of airway geometry in determining the specific anatomic deposition sites for these pollutants and the amounts that deposit at these sites. Because the anatomy of the respiratory tract varies widely among species, understanding the impact of airway geometry on tissue dose is particularly relevant for extrapolating data from laboratory animals to humans. The following section discusses recent work in this area and describes research needed in

the regional tissue dosimetry (pharmacokinetics) of formaldehyde and acetaldehyde.

Airflow Simulation

Recent improvements in computer technology have made it possible to simulate physiologic airflow with three-dimensional models of airways. Images of serial cross sections from selected regions in the respiratory tract can be digitized into a computer; special computer programs can then be used to reconstruct graphic representations of the airways from the digitized data. This approach has been used for anatomic models of the anterior nose of the rat (Kimbell et al. 1992, Miller et al. 1993). These computer models also can simulate airflow patterns similar to those observed in actual casts of the rat nose. The resulting data can be used to predict regional dosimetry of inhaled materials with a high degree of resolution. Extension of this approach to models of the human respiratory tract could provide simulation-based predictions for exposures in which humans receive regional tissue doses comparable those that produce cancer in rats.

Research Recommendations.

- *Research should be directed toward developing computer models of the respiratory tracts of humans and of those species of laboratory animals that are used in formaldehyde and acetaldehyde research, such as rats, rhesus monkeys, and, perhaps, mice. Anatomic variation within and across species should be considered.*
- *Factors that affect the uptake of the inhaled compound by target tissue cells could also be included in these models.*
- *Simulations of realistic breathing patterns could be used to describe regional uptake with particular emphasis on correlating uptake with measures of tissue dose, such as DNA-protein cross-links and with tissue responses such as cell replication, the formation of preneoplastic lesions, and tumor formation.*
- *This computer-based approach should be applied to predict human regional dosimetry and potential human carcinogenic responses to inhaled formaldehyde and acetaldehyde.*

Respirable Particles as Carriers for Inhaled Formaldehyde

Whether deposition of formaldehyde that has been adsorbed to particles produces greater effects in respiratory tract than formaldehyde vapor alone is a frequently raised question. Rothenberg and colleagues (1989) studied the surface area, adsorption, and desorption of formaldehyde on indoor dust. They predicted that the dose of particle-associated formaldehyde delivered to the respiratory tract would be at least four orders of magnitude smaller than the vapor-phase dose delivered to the upper respiratory tract. Similar findings have been reported by Risby and colleagues (1990).

The findings of these two investigator groups suggest that there is little likelihood that particle-associated formaldehyde could pose a greater risk than exposure to formaldehyde alone. However, they do not address the very different issue regarding the effects caused by simultaneous exposure to formaldehyde and particles. That question addresses whether joint exposure results in a synergistic effect that is greater than the simple additive effects of the two pollutants alone. Some epidemiologic evidence indicates increased effects from coexposure to formaldehyde and particles (Marsh et al. 1992).

DNA-Protein Cross-Links

Because formaldehyde and acetaldehyde form cross-links between DNA and protein, these reactions have been advanced as a potential mechanism for the cytotoxicity and carcinogenicity of these aldehydes (Lam et al. 1986, Casanova et al. 1989, Heck et al. 1989). However, to date, the primary application for DNA-protein cross-links (DPX) data in risk assessment has been as a measure of formaldehyde dose in tissues. Use of DPX as a tissue dosimeter does not depend on an understanding the mechanisms by which formaldehyde exerts its toxic and carcinogenic effects. Rather, the utility of DPX in risk assessment calculations rests on the assumption that they are a better measure of biologically effective dose than the concentration of the inhaled formaldehyde. The advantages and disadvantages of using DPX as a tissue dosimeter have been discussed extensively (California Environmental Protection Agency 1991, U.S. Environmental Protection Agency 1991).

Understanding of the basic chemistry of the reactions of formaldehyde and acetaldehyde with DNA can yield important information about the rates of DPX formation, their chemical stability, and about other products of cross-linking reactions. This information is critical for evaluating potential biomarkers of human exposure to these aldehydes and for a general understanding of how these aldehydes induce DNA damage.

Several areas of uncertainty exist regarding the reactions of formaldehyde and acetaldehyde with proteins and DNA that yield DPX. Much more detailed information is needed about the mechanism for this reaction. Although extensive work has been carried out on the initial interactions between formaldehyde and DNA (Feldman 1973; McGhee and von Hippel 1975a, b, 1977a, b) few mechanistic studies have been conducted on the ternary reaction involving formaldehyde or acetaldehyde with DNA and proteins. For example, the order of this cross-linking reaction, the nucleophiles involved, differences in reactivity among the DNA bases, and the hydrolytic stability of the DPX are all unknown. In addition, we need to characterize the protein aspect of the reaction. We know very little about the identity or relative distribution of the proteins involved in this reaction. Some data suggest that a particular histone is involved in the reaction between formaldehyde and DNA in Chinese hamster ovary cells (Miller and Costa 1989). It would be useful to know if this applied to rodents and primates.

The formation of DPX is nonlinear with respect to concentration (Lam et al. 1986). This nonlinearity has been attributed to saturation of a detoxification pathway, namely, the oxidative pathway from formaldehyde to formate or from acetaldehyde to acetate. Although little is known about the repair of DPX, repair may contribute to the observed nonlinearity of the dose-response curves for DPX and tumors.

It is not clear which enzyme or group of enzymes is primarily responsible for the detoxification of aldehydes. More information is needed about time-dependent changes in enzyme levels or activities as a function of aldehyde concentration during the course of cancer bioassays. In addition, nothing is known about the susceptibility of these enzymes to inactivation by these aldehydes.

The observation that cell proliferation occurs after exposure to high concentrations of aldehydes may be related to the extent to which aldehydes bind to DNA. Compared with DNA in resting cells, DNA in the process of replication during cell division is much more accessible for reactions with aldehydes or hydroxymethyl amino proteins. Because more DNA sites are available in this phase of the cell cycle, more cytotoxic DNA lesions could form, leading to increased cell death and enhanced cell turnover. This proliferative cell response and the subsequent increased exposure of sites susceptible to DNA cross-linkage could easily produce a nonlinear increase in aldehyde binding.

Research Recommendations.

- *More information is needed about the basic chemistry of aldehyde reactions with DNA and proteins.*
- *The potential roles of repair of DPX and changes in detoxifying enzyme activities as a function of time and of aldehyde concentration should be studied.*
- *Specific research should be conducted on the formation of DPX as a function of cell proliferation due to the possibility that DNA undergoing replicative synthesis may be more susceptible to attack by aldehydes.*

Mixtures

Aldehyde dehydrogenase has been recognized as a potentially important enzyme for the detoxification of both formaldehyde and acetaldehyde. This observation suggests the possibility of a synergistic toxic effect if an individual were exposed simultaneously to both compounds, as is likely in "real-world" exposures. In principle, because aldehyde dehydrogenase has a greater affinity for acetaldehyde than for formaldehyde, simultaneous exposure to both compounds could result in an inhibition of formaldehyde metabolism and an exacerbation of its toxicity.

Experiments with ozone and acrolein, two pollutants associated with motor vehicle emissions, have demonstrated that these compounds can enhance the toxicity of inhaled formaldehyde (Lam et al. 1985, Reuzel et al. 1990). Acrolein affects formaldehyde metabolism by depleting glutathione, a cofactor required for formaldehyde oxidation during catalysis by formaldehyde dehydrogenase. The mechanism by which ozone exerts its effect on

formaldehyde is not currently understood, but a mechanism similar to that of acrolein may be involved. These findings suggest that compounds other than acetaldehyde that are present in automobile exhaust or ambient air can affect the dosimetry and toxicity of formaldehyde.

The issue of chemical mixtures and the contribution of aldehydes to their toxicity is an important research area that should be addressed when considering research priorities for risk assessment. Studies in which formaldehyde and acetaldehyde are components of mixtures are more relevant to actual ambient air exposure conditions than studies in which each chemical is evaluated separately. A critical need exists to evaluate the dosimetry of formaldehyde during coexposures with other ambient air pollutants, such as acetaldehyde, ozone, or acrolein, that may exacerbate formaldehyde's toxicity. These studies will yield the most useful information if they are conducted with pollutant concentrations that are comparable to those in ambient air.

Research Recommendations.

- *Information is needed about the toxicology of formaldehyde as a component of chemical mixtures. Specifically, this information should contain data on formaldehyde dosimetry (currently assessed by DNA-protein cross-link formation) and toxicity (currently assessed by estimates of cell proliferation).*
- *Data should be obtained over a range of formaldehyde concentrations and should include both acute and subchronic exposures. Mixtures for initial consideration are formaldehyde plus acetaldehyde and formaldehyde plus ozone. These compounds are components of automobile exhaust or products of their atmospheric reactions and, thus, are plausible candidates for toxic interactions.*

Tissue Response

Squamous Metaplasia Squamous metaplasia has been reported in the nasal cavity of rats and monkeys after one month of exposure to formaldehyde (U.S. Environmental Protection Agency 1991). Squamous metaplasia, a reversible, adaptive response by the epithelium, indicates that the normal columnar epithelium has been replaced by one that is less susceptible to formaldehyde's toxic effects. If squamous

metaplasia is also an early preneoplastic event, then research is needed to address this issue. Tissue samples from rats and monkeys could be used to quantify the extent and severity of squamous metaplasia and to compare the responses between these two species. This approach presents an alternative to DPX measurements, which are currently regarded only as a measure of tissue dose. If existing tissue slides and blocks from previous studies could be used for these proposed metaplasia analyses, new exposure studies could be avoided.

Research Recommendations.

- *The role of squamous metaplasia in the pathogenesis of tumors induced by formaldehyde exposure should be evaluated. Specific attention should be directed toward determining whether squamous metaplasia is solely an adaptive response or whether it represents an early stage in a preneoplasia. If further research suggests that squamous metaplasia is one phase of a preneoplastic change, then quantitative assays should be done using this endpoint. Tissue samples from rats and monkeys could be evaluated using this endpoint to develop an interspecies comparison of formaldehyde's potency.*

Molecular Biology to Understand Mechanisms and Identify

Biomarkers Although formaldehyde has induced tumors in laboratory animals only when levels of exposure were 6 ppm and higher, concern for health effects from occupational and environmental exposure focuses on levels below 1 ppm. Practical constraints, such as the large number of animals that would be needed, inhibit the conduct of a bioassay that would be sensitive enough to characterize the shape of the formaldehyde exposure-response curve below 1 ppm with any certainty. Predictions of the shape of the curve at low levels of exposure therefore depend entirely on assumptions about the mechanisms that determine the shape of the curve at these levels.

Research Recommendations.

- Mutations in the p53 gene have been identified in cell lines derived from formaldehyde-induced tumors (Recio et al. 1992). These preliminary

results suggest that formaldehyde-induced lesions could be identified at the molecular level. This mutation could provide insights into the molecular mechanisms for formaldehyde-induced carcinogenesis and serve as a biomarker of exposure. Although it is not possible to identify formaldehyde-induced tumors using concentrations less than 1 ppm, it may be possible to identify these effects on a suitable biomarker in this low exposure range. Predictions about the shape of the exposure response curve at low levels of exposure might be possible using this approach and a combination of laboratory studies and simulation modeling. Parallel studies using molecular biology techniques for rodent and human tissues could provide valuable data for animal to human extrapolations. Although these are clearly longer-term research goals, they present promise for placing low-dose and interspecies extrapolation on a firm mechanistic footing.

Effect of Formaldehyde Exposure on In Vivo Mutation Rate Measured in the Transgenic Rat

Both formaldehyde-stimulated cell proliferation and DNA-protein cross link (DPX) formation are thought to be components of the carcinogenic mechanism of formaldehyde. Use of data on cell replication and DPX formation for health risk assessment would be facilitated if more were known about the relationship between these endpoints and the induction of mutation in vivo. Such data could be obtained using a transgenic rat in vivo mutagenicity assay.

Research Recommendations.

- Transgenic rats should be used to obtain in vivo exposure-response curves for formaldehyde-stimulated mutation, cell proliferation, and DPX formation in the nasal epithelium.

Models for Low Dose Extrapolation

Currently, the linearized multistage (LMS) model is the mathematical model most often used for extrapolation to low doses in cancer risk assessment. The LMS model is a modification by Crump and colleagues (1977) of the multistage model described by Armitage and Doll (1961). The

LMS model was developed in response to the recognition that cancer is a multistep process with procarcinogenic mutations responsible for movement from one stage to the next. Scientific understanding then and now frequently does not allow identification of the actual number of stages with any certainty. The current practice followed by the EPA is to utilize the LMS by setting the number of stages to one less than the number of dose groups in the bioassay. The LMS model is easy to use because, in the default mode, the only data required are the exposure concentrations or doses and the tumor results for each dose group. The model then calculates the maximum likelihood estimate of risk as well as the confidence intervals about this estimate. The LMS is considered conservative in its low dose extrapolations. This conservatism, combined with its ease of use, have contributed to the popularity of this computer model for cancer risk assessment.

As long as cancer risk assessment has involved no more than a listing of data for dose groups and tumor outcomes, the LMS model has served well. In recent years, however, it has become apparent that the carcinogenic process involves not only mutation but also cell replication (Cohen and Ellwein 1990). Data on the time course of cell replication have been obtained from a formaldehyde bioassay described by Monticello and associates (1991). The exposure-response curve for cell replication stimulated by formaldehyde correlates closely with that for formaldehyde tumorigenesis in the Fischer-344 rat. It seems reasonable that these data should be used in quantitative cancer risk assessment for formaldehyde. However, the LMS model has no provision for incorporating cell proliferation data. Thus, advances in mechanistic research have created a need for a cancer risk assessment model that includes not only the multistage nature of carcinogenesis but also cell proliferation within each stage.

Moolgavkar and coworkers (Moolgavkar and Venzon 1979; Moolgavkar and Knudson 1981; Moolgavkar and associates 1988) have developed multistage mathematical models for cancer. In the multistage clonal growth (MCG) models, cell division and cell death are described stochastically within each stage. A stochastic description—that is, based on random occurrence—is important when the number of cells in a given population is small. For example, when a cell in the first stage of a

multistage process mutates into a cell in the second stage of the process, a nonzero probability exists that it will die before dividing, even when the average division rate of cells in the second stage is greater than the average death rate. Thus, accurate description of the growth of populations with small numbers of cells requires a stochastic approach.

One difficulty presented by use of MCG models is that they are highly parameterized. Because laboratory estimates for all of the required parameter values are not easily obtained, the risk assessor is faced with the problem that several parameters remain unconstrained even when some of the parameter values are set against cell replication data. A statistical approach to resolving this problem has been described by Moolgavkar and Luebeck (1992). They fit MCG models with two and three stages to a data set on human colorectal carcinoma. The two models fit the data equally well, but they preferred the three-stage model because its parameter estimates were more biologically reasonable. Another application of MCG models, which has been used specifically for formaldehyde risk assessment, has been described by Conolly and colleagues (1992). These investigators have described a strategy for estimating all parameters in a two-stage clonal growth model from data on the pathogenesis of formaldehyde tumors. Because this approach for implementing an MCG model requires rich data sets, it is not currently feasible for most carcinogens. It is, however, useful for identifying data gaps and for research planning. It seems likely that a combination of these two approaches, in which a limited data set is used to fix some parameter values and statistical techniques are used to estimate the others, may be useful.

Research Recommendations.

- *Risk assessments for formaldehyde should be conducted using MCG models that incorporate statistical and data-based approaches to estimate the required parameters. The parameter estimates should then be used to identify the most plausible model. A more complete parameterization of this model from pathogenesis data could then be attempted. Estimates of risk at low formaldehyde doses should be more accurate with this model than those obtained with an LMS model. At present, there are*

insufficient data to support a similar approach for acetaldehyde.

NONCANCER HEALTH EFFECTS

Irritation

Considerable information is available regarding the potential for airborne formaldehyde to produce irritant effects in occupationally exposed individuals and in individuals in the general population. Formaldehyde has a characteristic odor that is noticeable at concentrations as low as 25 ppb, a level that is detectable by approximately 10% of the population. Concentrations of 170 ppb can be detected by approximately 50% of exposed individuals, whereas 1,500 ppb is detectable by all exposed persons (Pettersen and Rehn 1977). Although the capacity to detect formaldehyde odor varies widely among individuals, acclimatization may occur during exposure such that the formaldehyde odor is no longer noticeable.

Exposure to formaldehyde can rapidly produce irritation in the eyes and the upper respiratory tract. Formaldehyde at 30 ppb can induce eye irritation, evidenced by increased blinking, lacrimation, and conjunctivitis (Rader 1977). Irritation can progress to moderate and severe levels at 80 ppb (Wayne et al. 1977), and prolonged exposure at this level often becomes intolerable. At about 80 ppb, some people experience upper respiratory tract irritation, indicated by nasal secretion or dryness, increased nasal resistance, and throat soreness, whereas almost all exposed individuals experience this extent of irritation before a level of 2,000 ppb formaldehyde is reached (Weber-Tschopp et al. 1977; American Conference of Governmental and Industrial Hygienists 1992a, b).

Concentrations of formaldehyde measured in indoor environments and less frequently in outdoor environments clearly overlap those concentrations that produce odor and irritant responses in the general population. Individuals exposed to 100 to 300 ppb formaldehyde in their homes report symptoms of exposure more frequently than individuals exposed to equivalent concentrations in occupational settings (Ritches and Lehnen 1987, American Conference of Governmental and Industrial Hygienists 1992). Although smoking habits, socioeconomic status, preexisting disease,

and interactions with other pollutants are likely to affect physiologic responses to formaldehyde, these factors have not been evaluated systematically (World Health Organization 1989, National Research Council 1992).

Results from a study by Kane and Alarie (1978) suggest that interactions between aldehydes and other air contaminants can influence the extent of physiologic responses to formaldehyde. These investigators exposed mice to various combinations of formaldehyde and acrolein (mixture concentrations ranged from 120 ppb formaldehyde and 370 ppb acrolein, a rather high level, to approximately 9,000 ppb of each aldehyde) and measured the percentage decrease in the animals' respiratory rate. Their results indicated that formaldehyde and acrolein interacted in a way that suggested competitive antagonism between the two aldehydes. Responses to aldehyde mixtures or mixtures with other irritants common in polluted air (e.g., oxidants) warrant further investigation. This recommendation is supported by the frequency of complaints from residents in urban areas following exposure to complex mixtures of air pollutants.

The cellular and subcellular mechanisms by which formaldehyde produces its irritant effects are unclear. Formaldehyde can evoke changes in epithelial blood flow by a mechanism that depends on neurogenic pathways (Lundberg et al. 1984). Neuropeptides, such as substance P, are sensory neuromodulators that are released by sensory nerve stimulation; they may be important mediators of formaldehyde-induced irritation. Formaldehyde may also affect enzymes in the airway epithelial cells responsible for degrading neuropeptides, such as neutral endopeptidase, and, as a result, prolong formaldehyde's irritant effects. However, at present, the mechanisms of formaldehyde irritation remain undefined.

Research Recommendations.

- *Further research is needed to evaluate the physiologic responses to combinations of formaldehyde and other ambient pollutants at concentrations relevant to ambient levels. These mixtures should represent those commonly experienced by the general population and include compounds such as other aldehydes and ozone. The experiments should be designed to determine whether the effects of these mixtures*

produce additive, antagonistic, or synergistic responses.

- *Further work is needed to evaluate the cellular and subcellular mechanisms of aldehyde irritation. Issues to be addressed by these studies should include evaluation of low-level effects with concern about species differences when applicable.*

Sensitization

Immunologic responses to formaldehyde have been studied in animal systems and in human populations (Cosmetic Ingredients Review Expert Panel 1984, Imbus 1985, Feinman 1988). Topical application of high concentrations of formaldehyde can cause skin irritation and initiate systemic responses such as anaphylaxis or allergic reactions such as contact dermatitis. The mechanisms for these immunologic reactions remain unclear, but it appears that reactions to formaldehyde involve both immediate (antibody-antigen-mediated) and delayed (cell-mediated) hypersensitivity. In immediate hypersensitivity, formaldehyde acts as an incomplete antigen (hapten) by binding covalently to constituent proteins in the skin or blood and by forming new antigenic determinants. Antibodies to conjugates between formaldehyde and proteins from hemolyzed red blood cell membranes or from human serum albumin have been identified. They include immunoglobulin E, G, and M subtypes (Feinman 1988, Patterson et al. 1989). It should also be noted that antibodies to formaldehyde have been detected in hemodialysis patients that had been dialyzed using equipment that had been sterilized with formaldehyde. Delayed (cell-mediated) hypersensitivity is not as well documented as immediate hypersensitivity.

It remains unclear whether allergic reactions that involve the lungs can be initiated solely by inhalation exposures (Hendrick and Lane 1975 1977, Hendrick et al. 1982, Lee et al. 1984, Patterson et al. 1989). Guinea pigs exposed to up to 10 ppm formaldehyde developed dermal sensitivity, but in no case was pulmonary sensitivity established (Lee et al. 1984). Although documented cases are rare, both immediate and delayed pulmonary reactions have been evoked in persons with a history of previous occupational exposure (Burge et al. 1985, Nordman 1985). However, the magnitude of this potential problem remains unknown. In one study, an inhalation

challenge with formaldehyde produced marked pulmonary responses in 12 (5%) of 230 persons who had been referred to an occupational health clinic for evaluation of formaldehyde hypersensitivity. However, this information is difficult to apply to the general population because it is unclear how well this nonrandomly selected sample represents the working population from which it was drawn. In addition, details on the extent and route of previous formaldehyde exposures were not presented. These rare outcomes must, however, be considered when evaluating increased health risks to the general population because so many people are exposed to formaldehyde. Nevertheless, the potential seems remote that ambient (nonoccupational) inhalation exposure would produce allergic pulmonary response.

Research Recommendations.

- *At present, skin sensitization to formaldehyde has been well studied and pulmonary sensitization appears to be rare. No further studies are recommended in these areas at this time. However, little information exists on the potential for eye sensitization after aldehyde exposure. This may be a worthwhile research area because eye irritation is a predominant symptomatic response among individuals chronically exposed to formaldehyde.*

Pulmonary Effects

Few pulmonary function changes have been reported when either healthy individuals or individuals with asthma inhale formaldehyde during clinical testing (Leikauf 1992). However, the results of these experiments are based on exposures to high levels of formaldehyde (e.g., 3,000 ppb) and relatively short exposure times, sometimes as little as 20 minutes. In the case of ozone, another air pollutant, the adverse effects produced by low-level exposures become evident only after 4 to 6 hours of exposure. Results from studies with guinea pigs (Swiecichowski et al. 1993) suggest that when low levels of formaldehyde are used, longer exposures may also be needed to evoke pulmonary responses. In those experiments, extending the formaldehyde exposure from 2 to 8 hours lowered the threshold dose necessary to elicit functional changes. In addition, findings from an epidemiologic study indicate an increased prevalence of asthma and chronic bronchitis in

children living in homes with formaldehyde levels ranging from 60 to 120 ppb compared with children living in homes with formaldehyde levels less than 60 ppb (Krzyzanowski et al. 1990). These results provide further evidence that longer exposures to low levels of formaldehyde may be needed to induce changes in pulmonary function.

Research Recommendations.

- *Investigations of the cellular and subcellular mechanisms that are responsible for the pulmonary responses produced by aldehyde exposure are needed. Because formaldehyde may be a carcinogen, experimental exposures with humans may be difficult to perform. Alternatively, efforts to develop animal models or in vitro human systems may be useful.*
- *Confirmation of the epidemiologic findings described above would be worthwhile. Reevaluation of existing data bases concerning correlations between infant respiratory disease and exposure to environmental tobacco smoke or usage of gas stoves are suggested.*

Susceptible Populations

Some individuals in the general population have reported that they experience an enhanced susceptibility to chemical contaminants in the air. Their symptoms are diverse and appear to involve many systems. This syndrome has been termed multiple chemical sensitivity (MCS) or environmental illness and is thought to result from an usually high exposure to organic compounds (Fiedler et al. 1992). Automobile and diesel emissions are among the agents suspected to produce the symptoms that have been reported by these susceptible individuals.

The considerable controversy currently surrounding MCS is based on the lack of definitive list of symptoms and the absence of diagnostic criteria. Typical symptoms are headache, fatigue, loss of appetite, anxiety, arthritic-like complaints, and rhinitis. Diagnosis may be further complicated by the phenomenon of adaptation in which patients become nonreactive to environmental chemicals and therefore do not react to provocative challenges during clinical testing.

Some individuals have specific antibodies to complexes formed between formaldehyde and proteins. However, the relationship between the presence of these antibodies and symptoms is

unclear. For example, patients undergoing kidney dialysis can develop a high antibody titer for formaldehyde complexes because their dialysis treatment involved the use of tubing that had been sterilized with formaldehyde. However, these patients do not display the symptoms associated with MCS.

Other populations may also be at increased risk from formaldehyde exposure. Although documentation is limited, studies indicate exposures to ozone or environmental tobacco smoke may compromise the respiratory function of children (Samet et al. 1991). These findings suggest that children may also be more sensitive to the effects of formaldehyde exposure.

The influence of genetics on pollutant susceptibility has received little study; however, genetic differences among populations or individuals may predispose them to adverse responses following aldehyde exposure. For example, rapid elimination of aldehydes depends on specific enzymes, such as aldehyde dehydrogenase(s), glutathione, tetrahydrofolate, and NAD(P), as well as cofactors. Alterations in the metabolic pathways responsible for constitutive and inducible synthesis of these enzymes and cofactors may increase an individual's risk of adverse effects. Recent studies of ethanol metabolism have identified an NAD⁺-dependent aldehyde dehydrogenase isoform (restriction length polymorphism) that exhibits reduced activity in Asian and Native American populations. Following ingestion of ethanol, these individuals are able to metabolize this compound normally to acetaldehyde via alcohol dehydrogenase, the microsomal ethanol-oxidizing system, or catalase. Acetaldehyde should then be rapidly converted to acetic acid, but owing to a alteration in the aldehyde dehydrogenase enzyme in these populations, circulating concentrations of acetaldehyde are elevated. The resulting accumulation produces dysphoric and sympathomimetic responses that are evidenced by facial flushing, tachycardia, muscle weakness, and a rise in circulating catecholamines. At present, no information is available regarding the potential pathophysiologic consequences of this enzyme polymorphism with regard to aldehyde inhalation.

Research Recommendations.

- *Because the number of individuals who have reported symptoms of MCS is large, the prevalence*

and causes of MCS must be evaluated. Clearer definition and diagnostic criteria for this putative syndrome are also needed. Researchers should investigate potential causative agents, as well as exposure scenarios. Once endpoints are ascertained, an appropriate animal model could be developed and used to study agents, exposures, and concentrations needed for induction of disease and elicitation of symptoms.

- *Further studies are warranted to compare responses of children with those of adults. The contribution of aldehydes to frequency of respiratory symptoms also requires further study.*
- *The molecular basis of increased aldehyde susceptibility due to aldehyde dehydrogenase polymorphism needs further investigation. Transgenic mice and cell preparations from affected individuals should be developed to investigate possible responses in vivo and in vitro, respectively.*

Neurotoxicity

Formaldehyde can alter the olfactory epithelium in laboratory animals after long-term exposure. Behavioral tests of threshold olfaction and odor discrimination as well as neuroanatomic assays indicating reduced numbers of sensory cells have been observed in the ferret following 3 month exposures to 250 ppb formaldehyde (Kulle and Cooper 1975, Apfelbach et al. 1992). Species and age differences have been noted in this effect. These results suggest that formaldehyde can alter receptor neurochemistry, but details of the mechanism for such changes are lacking.

Formaldehyde exposures have been linked with an array of other neurologic symptoms including dizziness, nausea, apathy, headache, and sleep disturbances. For the most part, these reports have been anecdotal in nature. Only one study (Kilburn et al. 1985) assessed neurologic function in histology technicians, a population with an occupational history of formaldehyde exposure. The extent and severity of exposure to formaldehyde were estimated from histories gathered from the subjects about the duration of their daily exposures and the number of exposure-years. Although the testing indicated deficits in neurologic function, it was not possible to directly attribute these deficits to formaldehyde exposure because of several confounding factors, including

age, solvent exposure, self-selection, and lack of exposure profile.

Owing to an absence of animal and human data, it is not possible definitively to rule out formaldehyde's role as a causative agent of the nonspecific complaints that have been reported in the literature. Furthermore, formaldehyde may act as a contributing agent for neurologic effects when it is part of a complex mixture.

Research Recommendations.

- *Further investigation is warranted on olfactory effects following low-level (greater than 250 ppb) and extended (greater than 90 days) formaldehyde exposures. These studies should place an emphasis on determination of neurochemical mechanisms for the observed effects.*
- *Carefully controlled neurologic studies of humans exposed to formaldehyde are needed.*
- *Individuals who have been occupationally exposed to formaldehyde could serve as a useful study population because they provide a source of subjects that have received both continuous and intermittent exposures. Studies with monkeys, ferrets, or rats provide another approach for evaluating the effects of formaldehyde. These studies should not, however, be considered the only investigative approach because the nature of the human complaints is nonspecific and evaluating such complaints in animal models is difficult. Neurotoxicology screening studies in animals could also provide insight into hazard identification.*

Reproductive and Developmental Effects

The effects of formaldehyde on fetal development have been studied in both mice and dogs (Marks et al. 1980, Hurni and Ohder 1973). No evidence of teratogenic activity was reported in either study even though the doses that were used produced clear evidence of maternal toxicity. Although there have been no studies on the effects of formaldehyde on reproductive performance in animals, the high reactivity of formaldehyde, the lack of in vivo mutagenic activity, and the infrequency of exposures because of formaldehyde's irritant properties make the risk of adverse reproductive effects in humans quite low.

The teratogenic potential of acetaldehyde has been the focus of several studies (U.S. Environmental Protection Agency 1987b). Several studies in the rat and at least one study in the mouse reported positive effects. It is important to note that all studies concerning the effects of acetaldehyde on the fetus have administered the compound intraperitoneally, by mouth, or intra-amniotically. As a result, these studies have limited utility for assessing risks to the fetus caused by acetaldehyde inhalation. Several studies evaluating the effects of acetaldehyde on embryo cultures have demonstrated embryo lethality, growth retardation, and terata. These studies, because of the artificial nature of the test system used, are not useful for assessing risk. This is especially relevant to acetaldehyde because biotransformation of this compound to other metabolites is very effective. Thus far, no studies have evaluated the teratogenic activity of acetaldehyde after delivery by inhalation or by mouth. There are no *in vivo* studies of the effect of acetaldehyde on reproductive performance.

Research Recommendations.

- *Few studies have addressed the risks that acetaldehyde may present to fetal development or reproduction. Because there is well-documented evidence for such effects of methanol and its metabolite, formaldehyde, in vivo reproductive studies with an animal model, such as rats, would be useful to evaluate the effects produced by acetaldehyde.*

Mutagenicity

The mutagenicity of acetaldehyde has been studied in several different test systems. It has been shown to cause cytogenetic damage in a variety of test systems *in vitro*. Limited information is available on the mutagenic activity in whole animals, and the data appear inconclusive. Additional *in vivo* data would be useful.

Research Recommendations.

- *In vivo mutagenicity studies should be conducted with acetaldehyde using relevant routes of exposure.*

PRIORITIZATION OF RESEARCH RECOMMENDATIONS

Exposure Assessment

- *It is important to determine all of the exposure sources that contribute to the total human aldehyde burden, not just those that are due to motor vehicles. Many indoor sources of formaldehyde and other small aldehydes have been recognized; their contribution to the total concentration of aldehydes in the air is substantial. Because 90% of one's time is usually spent indoors, the indoor air exposures must be considered. In addition, the contribution of foods and cosmetics should be investigated. **High priority.***
- *The distribution of exposures to formaldehyde and other aldehydes within the population needs to be understood. For example, exposures may vary considerably among different age groups. **Moderate priority.***
- *The frequency and levels of peak aldehyde exposures should be examined. This information could be used to identify overlaps between the levels of environmental exposures and levels known to be toxic to laboratory animals and humans. **Moderate priority.***

Carcinogenicity

- *A new metaanalysis of the existing formaldehyde epidemiology data is recommended that would include analysis of the original exposure and incidence data. Ideally, such an analysis would establish a quantitative exposure-response relationship for humans exposed to formaldehyde and yield a cancer potency value. A less ambitious, but nonetheless important goal for this study would be to identify plausible constraints on the potency estimates that have been used to extrapolate the data from animal exposure studies to human exposure scenarios. **High priority.***
- *The role of squamous metaplasia in the pathogenesis of tumors induced by formaldehyde exposure should be evaluated. Specific attention should be directed toward the question whether squamous metaplasia represents an adaptive or preneoplastic*

change. If additional research suggests that squamous metaplasia is a preneoplastic change, then this endpoint can be used for evaluating tissue samples from rats and monkeys and for developing an interspecies comparison of formaldehyde's potency. If existing tissue slides and blocks from previous studies could be used for these proposed metaplasia analyses, new exposure studies could be avoided. *High priority.*

- The chemistry of DNA-protein cross-link (DPX) formation after exposure to formaldehyde or acetaldehyde and the usefulness of DPX in understanding the dosimetry of aldehyde mixtures require further examination. A study of the interactions between aldehydes is important for understanding use of DPX as a biomarker of exposure. In addition, the biological consequences of DPX formation require clarification. *Moderate priority.*
- Additional study of the factors that influence the dosimetry of inhaled aldehydes in the respiratory tract is needed. One example is the impact of airway geometry and varying ventilation levels on the specific sites of deposition for the inhaled vapors. Such information is necessary for understanding species differences in toxicity and understanding intraspecies variation in outcome. *Moderate priority.*

Noncancer Health Effects

Irritation

- Dose-response studies of the effects of mixed exposure of aldehydes with other airborne chemicals to which the population is commonly exposed, such as ozone, are recommended.
- The process of adaptation must be understood. To this end, the cellular and subcellular mechanisms of aldehyde irritation should be evaluated. Issues to be addressed by these studies should include evaluation of low-level effects with concern about species differences when applicable.

Pulmonary Effects

- Investigate the cellular and subcellular mechanisms associated with aldehyde effects

on pulmonary responses. Due to ethical concerns about exposing humans to probable carcinogens, development of animal models or human *in vitro* systems may be useful.

- Reports of increases in asthma and bronchitis among children living in homes with increased formaldehyde levels suggests that further study of these observations is needed. Because environmental tobacco smoke has been linked to an excess of 300 to 1500 cancer deaths and to more than 100,000 cases of respiratory function alterations among children per year, morbidity studies may provide more meaningful data than mortality studies. These data are particularly relevant to aldehydes because environmental tobacco smoke contains high levels of aldehydes and produces similar patterns of adverse effects, such as eye and throat irritation.
- Transgenic rats should be used to obtain *in vivo* exposure-response curves for formaldehyde-stimulated mutation, cell proliferation, and DPX formation in the nasal epithelium.

Susceptible Populations

- The molecular basis of increased aldehyde susceptibility due to polymorphisms in aldehyde dehydrogenase needs further investigation. Transgenic mice or cell cultures obtained from affected individuals should be developed to investigate potential *in vivo* and *in vitro* responses.

Neurotoxicity

- Olfactory effects produced by aldehyde exposures require further investigation. This is particularly important because anosmias, such as those induced by exposure to isobutyraldehyde, are very common and yet rarely reported by the general population.

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Research Priorities to Reduce Uncertainties in Risk Assessment for Benzene

INTRODUCTION

Benzene toxicity has been documented on the basis of observations in humans exposed in the workplace. Some aspects of benzene toxicity have been observed in animal models. Recent reviews of the literature on benzene include Snyder and Pirozzi-Chatterjee (1991), Pirozzi-Chatterjee and Snyder (1991), Yardley-Jones and associates (1991), and Snyder and associates (1992). Ultimately there are three documented outcomes of human exposure to benzene: pancytopenia, chromosome aberrations, and leukemia. Pancytopenia in its most severe form is known as aplastic anemia, a syndrome characterized by decreases in the numbers of circulating red and white blood cells and platelets, accompanied by the almost complete replacement of many bone marrow cell types by fat and scar tissue with relatively few original cells remaining active (Pirozzi-Chatterjee and Snyder 1991). Aplastic anemia is known to require prolonged exposure to high levels of benzene. Chromosome aberrations such as breaks (Forni et al. 1971a,b), sister chromatid exchanges (Tice et al. 1990), and micronuclei (Hite et al. 1980) may be considered markers of exposure to benzene as well as markers of effect; also, because damage to chromosomes can potentially lead to adverse outcomes, they may also act as markers of susceptibility. Therefore, thorough understanding of the relationship of chromosome damage to hematopoietic toxicity and cancer may provide approaches to improve our evaluation of dose-response relationships. Finally, benzene exposure in the workplace has been shown to result in acute myelogenous leukemia (Infante et al. 1977).

These effects are known to result from exposure to the high levels of benzene that were

previously prevalent in the workplace. The critical issues of concern for this document are whether any or all of these effects can also be produced at low ambient environmental levels of benzene, and the dose-response relationship at these low levels. People who have been treated with anticancer alkylating drugs and who experience damage to their bone marrow may serve as a potential model of the relationships between benzene and acute myelogenous leukemia (Goldstein and Kipen, 1990). As with benzene, there may be many degrees of bone marrow damage, ranging from a decrease in any of the circulating cell types, or in more than one cell type, to pancytopenia with or without complete marrow aplasia. A significant number of cancer patients successfully treated with alkylating drugs eventually develop acute myelogenous leukemia. Thus, one eventual outcome of recovery from aplastic anemia can be acute leukemia.

This chapter seeks to identify what research is needed to determine if leukemia can result from bone marrow effects less severe than aplastic anemia. For example, can slight decreases in circulating cells, resulting from mild bone marrow damage, be indicative of a risk of eventual leukemia? Is an individual exposed to benzene in the general environment who shows no signs of decreases in circulating cells at risk of leukemia as a result of benzene exposure?

This chapter is organized to consider research needs in several areas relevant to minimizing uncertainties in benzene risk assessment. These areas include: (1) exposure assessment, (2) epidemiology, (3) experimental animal models for carcinogenic effects, (4) metabolism and mechanisms, (5) genetic toxicology, (6) risk assessment, and (7) dose-response modeling. In addition, a final section summarizes general research needs for the development of biologically based dose-response models for benzene and other airborne toxics.

EXPOSURE ASSESSMENT

In 1985 the U.S. Environmental Protection Agency (EPA) identified major categories of benzene sources in the environment and concluded that approximately 85% of all benzene emissions originate from combined exhaust, evaporative, and

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refueling emissions from motor vehicles. Coke by products, fugitive emissions, and gasoline marketing make up most of the remaining 15% (General Accounting Office 1983). Benzene emission inventories may, however, vary in individual states and cities depending on what industries are nearby.

Benzene in ambient or outside air, however, may not be the most important source of human exposure for several reasons. First, many people spend most of their time indoors. Indoor concentrations of benzene have been found to exceed outdoor concentrations up to several fold (Wallace 1989). Second, concentrations of benzene in cigarette smoke are much higher than in the ambient air. Third, people may ingest benzene or benzene precursors from the food they eat. Fourth, a variety of consumer products and activities within the home can substantially contribute to benzene exposure. Stored gasoline, lawn mowers, and many other benzene-containing products can be found in most homes. Home furnishings, including paints, adhesives, and inks, might also contribute to the benzene exposure burden. In 1989, the EPA Total Exposure Assessment Model studies found that although motor vehicles are credited with 85% of the benzene emissions to ambient air, the real motor vehicle contribution to personal benzene exposure is only about 20% because a much larger fraction of exposure, 56%, is due to cigarette smoking and 21% to home activities (Wallace et al. 1989). For these reasons, an estimate of the total daily intake (both average and time-based profiles) of benzene would be a better measure of human exposure than are ambient air concentrations. Exposure research should focus on determining the total personal exposure resulting from a variety of sources in and around human microenvironments.

Issues and Uncertainties

The most pressing exposure research needs are in the area of the integrated personal exposure assessment, particularly of specific microenvironments. Given that Americans spend most of their time indoors, indoor microenvironments and the associated human activities need better characterization.

Exposure involves more than just toxin concentration. For exposure to occur, people must be present where there is a toxin. The various

activities of people in an indoor environment have a great impact on their exposures. Individuals' activities affect their own exposure, as well as the exposure of others. Activities which affect breathing rates also impact on benzene uptake into the body. Thus far, most activity studies have been time and location studies. Further investigation is needed to better understand the important subsets of activities that have an effect on the ultimate exposure. It would also be useful to find ways to incorporate more mechanistic information into the current stochastic models of exposure, such as the EPA's Benzene Exposure Assessment Model (BEAM). Also, carefully designed monitoring studies are needed both for the validation of current models such as BEAM, and for the development of newer and improved models (including mechanistic models based on thermodynamic principles) of microenvironmental exposure. Models of indoor air should be able to account for benzene exposure resulting from active and passive smoking.

The potentially significant contribution to benzene exposure from emissions within attached garages needs to be accurately assessed. The conditions under which the contribution is greatest and the sources (evaporative emissions from automobile engine cooling, the automobile gasoline tank, stored gasoline, and other consumer products) need to be elucidated. Continued evaluation of the situation as emission and control devices are placed on future automobiles is also needed. Studies are needed to determine what conditions of the home and its construction can be modified to reduce the transfer of benzene vapors into the living areas. The research should be designed to investigate seasonal and other variations that may affect exposure. It is not recommended that large, nationwide monitoring studies be attempted. Rather, a program of research using models (both currently existing and new, more sophisticated mechanistic models), interfaced with carefully designed monitoring studies as necessary, should be implemented. With careful planning, such a program could be in place within a short time and yield a great deal of information about in-home exposure to benzene.

With both the activity and emission models described in preceding paragraphs, assessors will be able to inexpensively and quickly test ideas about theorized or observed exposure scenarios and even test the usefulness of possible

amelioration strategies. Properly used, models serve to identify the focus of any needed monitoring studies.

Many consumer products are known to contain benzene and emit its vapors, but more testing and modeling are necessary to determine the exact contribution of these products to the indoor air exposure burden. Such materials commonly found in the home include paints, adhesives, and rubber products. The role of furniture and carpet materials that might serve as sinks for benzene and be sources of later emission needs to be further elucidated.

Pharmacokinetic Models for Dose

Assessment Exposure assessment, to be most useful, should include dose assessment. Ultimately, risk assessment is based on the internal dose at the target site. The actual measure of effective dose can be the parent compound, specific metabolites, or combinations of all (parent and metabolites), depending on the toxic endpoint of interest. Pharmacokinetic models are effective tools for calculating the relevant target dose for use in risk assessment. These models will also be very useful for determining the importance of various exposure scenarios on the ultimate target dose. As such, they can be useful in helping to plan what type of exposure models and exposure monitoring studies are needed. With proper physiologic information, a pharmacokinetic model can be easily used to compare the impact resulting from one route of exposure with that from other routes. As discussed in other parts of this document, for the model to be effective, more needs to be known about the mechanism of action, metabolism, and so forth. Research is needed in these areas, but this work should be closely coordinated with modelers who can develop higher resolution pharmacokinetic models as data are developed through the mechanistic studies. The interesting models published by Medinsky and colleagues (1989) need more extensive validation and expansion for use in human risk assessments.

Biomarkers Biomarkers of exposure are becoming increasingly available for benzene. Examples are muconic acid in the urine, various DNA and protein adducts, as well as exhaled benzene itself. Properly tested and formulated pharmacokinetic models are necessary to estimate exposure from biomarker data. In addition, there

is a great need for the development of advanced mathematical techniques for the proper, efficient, and accurate solution of the models to estimate exposure. It may be necessary to use a suite of biomarkers for benzene exposure assessment. Some markers may reflect recent exposure (as, for example, muconic acid); others may represent more integrated measures of past exposure (as, for example, hemoglobin adducts). In this manner the unique time-based pharmacokinetic profile of each marker can be used to help derive exposure estimates from biomonitoring data. It should be remembered that concentrations of biomarkers depend on complicated physiologic and biochemical processes. As such, there can be a great deal of physiologically dependent variability between people and even within the same person. Exposure assessors using biomarker-based techniques will need tools to distinguish between differences in marker levels due to peculiar (or variable) metabolism and physiology and those due to true differences in exposure. In addition to the variance expected in the normal population, care should be also taken to have tools that can account for those individuals with specific conditions such as diabetes that cause additional variation in metabolism beyond the expected variance in the normal population.

Research Recommendations.

- Improved characterization and modeling of human indoor air exposure. Exposure variations due to activities, age, location, and so forth, should be evaluated (such research would aid the exposure assessments of chemicals other than benzene as well). Monitoring (videotaping, surveys, questionnaires, and so forth) should be performed as needed to help model building.
- *Improved and more mechanistically detailed microenvironmental models for the dispersion of pollution throughout the indoor air environment.* Some of the information gained here will also be relevant to exposure assessment of other pollutants. Monitoring should be done only to support model building, testing, and validation.
- *Study of interindividual variation.* It is generally accepted that the first step in benzene metabolism is catalyzed by cytochrome P-450 2E1 (Koop et al. 1989, Chepiga et al. 1991). It is also known that this P-450 isoenzyme is polymorphic in humans (Hayashi et al., 1991), with different allelic frequencies among

different racial groups (Kato et al., 1992). Thus there could be detectable populations or ethnic groups that may be at increased risk of benzene's effects. The cytochrome P-450 2E1 polymorphism can be detected by a genotyping method based on the polymerase chain reaction (PCR) or by the drug chlorzoxazone. These approaches should be employed in studies of benzene-exposed populations in a pilot study. If sensitivity may, in part, be detected by this method, then epidemiologic studies should be conducted on sensitive individuals within an exposure group.

- *Implementation of current pharmacokinetic models and the development of pharmacokinetic models that reflect the more detailed metabolic and mechanistic information as it develops.* Focus should be placed on facilitating interactions with biochemical and mechanistic researchers. A model with a representative bone marrow compartment should be a high priority. In addition, the animal model should contain the Zymbal gland as a compartment. This could be important for future risk assessment purposes if the benzene-induced chromosomal changes in rodent Zymbal gland are similar to those detected in human leukemias.
- *Continued and expanded research on developing a suitable battery of biomarkers.* In addition to the analytical techniques, the associated pharmacokinetic information for each biomarker should be developed. Such information should then be put into the pharmacokinetic models.

EPIDEMIOLOGY

A number of studies of benzene-exposed populations have been completed (Austin et al. 1988). Aksoy (1976), in a study of 31 leukemia cases, estimated the leukemia rate to be 13 per 100,000 person-years among workers exposed to benzene compared with the estimated rate of 6 per 100,000 person-years among males in the general populace. Vigliani (1976) estimated the incidence of leukemia to be 20 times higher among benzene-exposed shoeworkers in Italy than among the general populace. Case-control studies of patients with hematologic disorders in France (Girard and

Revol 1970) and of Japanese patients with leukemia in Hiroshima and Nagasaki (Ishimaru et al. 1971) found elevated risks for benzene exposures. Several interesting cohort studies of benzene workers who were exposed or potentially exposed to benzene have been completed (Infante et al. 1977; Ott et al. 1978; Rinsky et al. 1981, 1987; Decoufle et al. 1983; Wong et al. 1983; et al. 1989). An in-depth assessment of most of these and other studies has been published by Austin and associates (1988) and will not be repeated here. In general, most studies of benzene-exposed workers have found increased risks of leukemia, particularly acute myelogenous leukemia. Solid tumors and possibly lymphoma may also be increased in benzene-exposed workers (Yin et al. 1989). The issue of whether benzene can cause an increase in acute lymphatic leukemia, the form of leukemia that is much more common in childhood, cannot be addressed in worker studies since children are not in the workforce. This remains a subject of particular concern because of community exposure to benzene.

One of the more controversial studies in terms of the content of exposure is the investigation of Pliofilm workers reported first by Infante and associates (1977) and later followed up by Rinsky and associates (1981, 1987). The results of Rinsky and associates (1987) are summarized in Table 1.

The primary point of contention concerns the manner in which exposures were estimated by Rinsky and associates (1987) and the impact these exposure considerations have on estimating the risk by exposure level. More recent estimates (Crump and Allen 1984, Paustenbach et al. 1992, Kipen et al. 1988,) have suggested that Rinsky underestimated exposures; if so extrapolations from the Rinsky data would overestimate the risk at low exposure levels. This issue has been explored by Paxton and associates (1993 a,b).

Results from a follow-up study of a large population of benzene-exposed workers in China were reported by Yin and associates (1989). This investigation also reported increased leukemia (SMR = 574) among those exposed to benzene. However, in addition to leukemia, lung cancer was also increased, especially among nonsmokers (relative risk = 231).

Table 1. Summary of Rinsky Study^a

Benzene Cumulative Exposure (ppm-yr) ^b	Time-Weighted Average	Observed	Standardized Mortality Ratio	95% CI
0.001-40	< 1	2	109	12-394
40-200	1-5	2	322	36-1,165
200-400	5-10	2	1,186	133-4,285
> 400	> 10	3	6,637	1,334-19,393
TOTAL	-	9	337	154-641

^a Also reported in Landrigan and Nicholson (1992).

^b Assuming exposure received over a 40-year period.

Issues and Uncertainties

Despite the considerable amount of epidemiologic and related research that has been completed, a plethora of important research issues remain. These include concerns related to exposure and dosimetry, health endpoints, other biological endpoints, confounders, and methodology.

Exposure and Dosimetry Many of the studies of workers have benefitted from area and individual monitoring for benzene levels. However, historical exposures usually have to be reconstructed from a variety of occupational records. These reconstructions require assumptions to be made that can engender considerable controversy, such as the debate regarding the exposure estimates used by Rinsky and associates in the National Institute of Occupational Safety and Health (NIOSH) study of Pliofilm workers (Paustenbach et al. 1992).

The fact that recent exposure guidelines have helped to decrease exposures complicates the process of identifying populations that have been sufficiently exposed to provide a range of exposed individuals to be studied. Populations that have been exposed only during the past several decades are unlikely to have large cumulative exposures. Hence, the primary type of exposure that will be encountered is to low levels. To be able to see an exposure effect, it is necessary to study large populations that can be followed for extensive periods of time.

Exposure issues become even more complicated when ambient environmental exposures or exposures encountered by the general

populace are considered. Problems of very low exposures that are difficult to measure increase the difficulty of designing epidemiologic studies that have a reasonable chance of detecting an effect, if an effect is present. It may also be difficult to reliably quantify interindividual variation in benzene exposure of a control population that results from a wide variety of sources (Wallace 1989). Identification of special exposure situations, such as exposures received while patronizing self-service gas stations, or encountered in homes where fuel oil is used for heating, or where gasoline-powered equipment such as chain saws or lawn mowers are routinely used, may be of some merit. Indoor exposures, such as those experienced from the indoor combustion of natural gas or exposures that are experienced from vehicles parked in attached garages, may deserve further consideration.

Health Endpoints The primary health endpoint that has been considered in most epidemiologic studies of benzene effects is acute nonlymphocytic leukemia. In addition, recent findings of solid tumors among benzene-exposed cohorts (Yin et al. 1989) indicate that benzene may induce neoplasms other than leukemia or blood cancers. Finally, the lack of information regarding the relationship of low-level benzene exposure to blood dyscrasias other than leukemia and to other noncancer outcomes constitutes a gap in our knowledge.

Other Biological Endpoints Techniques of molecular biology are developed to a point where

application of biomarkers to the study of human populations that are exposed to low levels of benzene may be important. More consideration needs to be given to the applicability of biomarkers such as chromosome breaks, DNA adducts, and known benzene metabolites to studies of human populations. Such applications may help to overcome the problems of extremely large populations and long follow-up times that are inevitable with low-level exposures.

Confounders As is the case with most epidemiologic research involving occupational or environmental exposures, the influence of important confounders is usually inadequately assessed and controlled. The effects of confounders such as mixed workplace exposures (often encountered by rubber and petrochemical workers) are unknown, as are the effects of medical exposures, lifestyle factors (smoking or substance use), and certain susceptibilities such as blood abnormalities.

Methodologic Issues A variety of methodologic issues need to be considered. The uncertainty of most risk estimates, as evidenced by wide confidence intervals, is a limiting factor that will become less of a concern with continued follow-up of previously studied populations. The appropriateness of conducting metaanalyses to decrease some of the statistical uncertainty needs to be considered. The use of poor or debatable exposure estimation techniques requires further consideration and rectification. Future risk estimates need to take into account biological interaction from multiple exposures to determine if synergistic relationships exist between benzene and exposures to other toxins.

A final methodologic issue concerns the appropriateness of applying the results from occupational studies to the general population. It is well known that worker populations differ in health status from the general population. Because of the greater numbers of susceptible individuals in the general population, uncritical application of risk estimates based on occupational studies to the general populace may result in an underestimation of the general population risk.

Research Recommendations.

- *Continued follow-up of worker cohorts.* Continued follow-up of worker cohorts that have already been studied is important for resolving some of the important issues that remain regarding risks associated with benzene exposures. Continued refinement of exposure estimates with the application of additional relevant data will help to better define the relationship between exposure and leukemia. In addition, continued follow-up of worker populations will permit estimation of risk for other health endpoints, such as multiple myeloma, non-Hodgkin's lymphomas, and solid tumors. If these tumors require longer induction times than leukemia, increased mortality for these tumors may now begin to be detected. Despite the controversy regarding exposure estimates, it should be remembered that workers are, on the average, still the best characterized and most identifiable group of individuals with known benzene exposures that can be readily characterized. Continued follow-up should also help to reduce some of the statistical uncertainty that exists for certain categories of workers.
- *Cohort studies of heavily exposed populations.* Because of the exposure standards that have been implemented in the United States, it is unlikely that populations that are exposed to relatively high levels of benzene will be identified. Populations that are still exposed to levels of benzene that exceed U.S. standards may be found in other countries. Cohorts have been identified and are being studied in China, and these studies need to be continued. Exposed populations also may be found in other countries such as Turkey, Poland, and other Eastern European countries. Of special interest are the populations who reside near and are employed by the Maquiladora industries on the Mexico side of the U.S.-Mexico border. Major concerns have been raised regarding occupational and environmental exposures associated with Maquiladora operations across the border from Brownsville and El Paso, TX, and other U.S. cities. Followup of worker populations in such areas may allow a prospective component to be implemented to pursue questions such as the relationship between exposure, effects manifested by various biomarkers, aplastic anemia, leukemia, and other potential health outcomes.
- *Case-control studies.* Case-control studies of selected health endpoints identified from

follow-up studies or experimental studies of animals are also needed to resolve yet another constellation of issues. Depending on circumstances, case-control studies nested within existing cohorts may be conducted, if large enough numbers of cases can be identified, and case-control studies that cut across several worker populations also would be possible given cooperation between relevant parties. Such studies would have the advantage of considering the relationship between benzene exposure and other exposures, or treating other exposures as confounders and controlling for their influence. An advantage of case-control studies is that they afford the opportunity to collect data on a number of exposure, lifestyle, demographic, and other variables for a limited number of cases and a sample of controls. In this way, confounders and effect modifiers may be assessed and appropriately treated at the analysis stage. Another advantage would be the additional resources that could be devoted to deriving improved exposure estimates for a much smaller number of study subjects than is possible with cohort studies.

- *Studies of environmentally exposed populations.* Investigations of employed populations have the disadvantage that such study results cannot be extrapolated directly to the general population without exercising considerable caution. It is well known that employed populations differ from the general population in a variety of ways. Workers are on the average healthier, receive better health care, and experience higher exposures than do members of the general populace. Therefore, studies of exposures that more closely represent the environmental exposures experienced by the general populace and include populations other than workers are needed to understand the impacts, if any, of environmental benzene exposures. Investigations of indoor exposures, such as occur in houses with attached garages, and studies of populations that reside in the vicinity of point sources or mobile sources of benzene exposure may meet this need. Further efforts need to be expended to identify other populations that are suitable for investigating the potential effects of ambient exposures. Cross-sectional, cohort, and case-control study designs may be applicable depending on the

exposure and on the health endpoint that is selected. Uncertainties include the feasibility of adequately quantifying exposure and identifying populations with detectable levels of exposure. These uncertainties may be partially overcome by implementing studies of populations in other areas, such as China, Mexico, and Eastern Europe, that reside in the vicinity of industrial facilities that emit benzene at levels exceeding U.S. standards.

- *Biomarker studies and noncancer health endpoints.* Most of the large epidemiologic studies of benzene exposures have focused on cancer mortality. Noncancer health outcomes and studies using biomarkers may be especially appropriate for questions regarding environmental exposures to mobile air toxics such as benzene. Noncancer health events such as blood abnormalities may be more important for assessing the effects of ambient benzene exposures than is mortality from leukemia and other cancers. Biomarkers carry the advantage that long latent periods are not necessary to observe an effect (although there may be some question whether the biomarker is measuring a health effect or simply an exposure effect). In addition, some biomarker studies, such as those of chromosome aberrations, can be conducted on much smaller populations than can traditional epidemiologic studies of health outcomes, because a large number of cells can be collected from a relatively small number of study subjects. Often, available or pertinent human study populations are not large enough to study health outcomes with any degree of desirable statistical precision.
- *Metaanalyses.* Studies of combined worker populations using metaanalytic techniques may allow more precise risk estimates to be calculated if the assumptions and requirements for combining different study populations can be satisfied. When appropriate, metaanalyses allow further analyses to be conducted on existing combined data sets without collecting more data. Such studies can be completed relatively quickly and inexpensively.

ANIMAL CANCER

In experiments with laboratory animals, benzene is a multisite carcinogen, and it is a carcinogen in both sexes of rats and mice. A wide variety of tumor sites were detected including Zymbal gland, lung, harderian gland, mammary gland, preputial gland, forestomach, ovary, skin, and oral cavity (Huff et al. 1989; Huff 1992). Moreover, increased lymphoma incidence has been observed in mice. The most complete bioassay was conducted by the National Toxicology Program (NTP). The route of exposure was gavage, which has raised the criticism that the inhalation route should have been used because it is a better simulation of the large majority of human exposures. However, an inhalation study by Maltoni and colleagues (1989) demonstrated that benzene is a multisite carcinogen by this route as well, although, in agreement with the NTP study, leukemia was not an outcome. For these reasons, we are not recommending that a full-fledged bioassay (both sexes of two species) be conducted. Rather, we encourage research to identify a suitable animal model for evaluating leukemia risks in humans exposed to low levels of benzene.

Rats are more resistant to benzene toxicity than mice. Some strains of mice such as C57BL/6 are more resistant to benzene toxicity than others such as DBA/2n (Longacre et al. 1981). Benzene toxicity in bone marrow is thought to require prior metabolism in liver, with subsequent transport of metabolites to the bone marrow. Although it is suspected that the difference in sensitivity between species has a metabolic basis, it has also been suggested that differences in bone marrow sensitivity to benzene metabolites may play a role. More studies are needed to determine the basis for sensitivity or resistance to benzene-induced bone marrow depression in various animal models.

The study of benzene-induced leukemia has been hampered by the lack of availability of appropriate animal models despite many attempts. Although there is agreement that availability of such models would permit the design of critical experiments, there is disagreement regarding the chance of success. In the absence of proper models, it has been impossible to study the mechanism of benzene-induced leukemia. Recent reports on benzene metabolism in monkeys and chimpanzees suggest that these species may serve as models for the human disease (Sabourin et al.

1992). Examination of strains of mice or rats not yet examined is also important. In addition, it will be important to examine hamsters, guinea pigs, and other species to determine whether better models might be developed.

Research Recommendations.

- *Research to develop an animal model for benzene-induced leukemia in humans.* This research should begin with attempts to validate an intermittent model similar to that of Cronkite and associates (1989) who demonstrated that intermittent exposures to benzene produced myelogenous neoplasms in CBA/Ca mice. A more recent study using a similar intermittent exposure regimen was not successful in repeating the original Cronkite findings of leukemia (Farris et al. 1993); however, the promise and the mechanistic plausibility of the Cronkite model for benzene-induced leukemia in humans justifies further study.
- *Research to evaluate the suitability of the Zymbal gland for estimating human cancer risks from benzene.* These studies should focus on comparative aspects of benzene metabolism and chromosomal changes in human leukemias and rodent Zymbal gland. Although a relevant animal model for benzene-induced leukemia would provide numerous opportunities to conduct research aimed at addressing uncertainties in risk assessment, it may be possible to use surrogate sites. For example, the Zymbal gland is the most consistent site for benzene-induced cancer. If the same chromosomal aberrations are observed in Zymbal gland tumors as are seen in benzene-induced leukemias, then the Zymbal gland may be a reasonable model to use in human risk estimates and the development of dose-response models.
- *Research to determine if aplastic anemia is an essential step in benzene-induced leukemia.* One of the most important knowledge gaps that limit our ability to develop reliable dose-response models for benzene-induced leukemia is understanding of the cellular mechanisms responsible for leukemia, especially those involved in the early stages. One possibility is that the development of some degree of aplastic anemia is a necessary step in leukemia. This mechanism would imply a different dose-

response relationship than if leukemia could develop without blood cell toxicity.

- *Research to elucidate the mechanisms responsible for benzene-induced solid tumors.* Benzene produced cancers at a number of sites in laboratory animals. If there is good evidence that benzene is a multisite carcinogen in humans, then risk assessments in humans will be more reliable if mechanistic information is available for the nonleukemia sites.

MECHANISMS

A full understanding of the adverse effects of benzene in the workplace, the home, or the general environment would require clarification of the underlying biological mechanisms by which chemicals harm people. Attempts to set acceptable standards for exposure to chemicals in various media, under a variety of circumstances, have often led to controversy because of our lack of sufficient information on underlying mechanisms. There is now a large body of literature concerning to studies of the mechanism by which benzene produces aplastic anemia (Snyder et al. 1992). Although the problem is not solved, an excellent beginning has been made to understanding how exposure to high doses of benzene can produce marrow aplasia. Whether or not these mechanisms apply at low environmental doses is unknown.

Studies in animals have suggested that chromosome damage may result from exposure to low doses of benzene (Meyne and Legator et al. 1980; Snyder et al. 1992). This damage has been postulated to play a role in the eventual production of leukemia. The mechanism by which these forms of damage occur must be examined, and we must explore the potential biological consequences of these forms of damage under ambient environmental conditions if we are to appreciate the impact of low-level exposure to benzene in the environment.

Finally, the question of greatest interest in this field is whether low-level environmental exposure to benzene can result in leukemia. If we argue that high-level exposure leads to some degree of marrow aplasia and that leukemia is a product of the recovery from aplasia, then levels of benzene that do not cause aplastic anemia should not produce leukemia. Alternatively, subtle alterations

in bone marrow function, even at low doses, may be sufficient to produce leukemia by mechanisms other than full-blown aplastic anemia.

Thus, mechanistic studies on benzene-induced bone marrow damage are essential to understand the environmental impact of benzene on humans. The following series of studies, in several categories, should lead to a better understanding of the mechanisms of benzene toxicity and leukemogenesis. In all of the studies suggested below, it will be essential that the impact of dose be critically evaluated. Ultimately it is important that methods be developed to use these data in the modeling and risk assessment process.

Research Recommendations.

Studies of effects on membranes. Benzene is known to produce general anesthesia and sensitization of the myocardium to catecholamines via an effect on cell membranes produced by benzene itself, rather than by its metabolites (Snyder and Kocsis 1975). Studies by da Silva and colleagues (1989) suggested that benzene enhances protein kinase C activity in cell membranes.

- *Because it may be a mechanism for promotion of benzene-induced cancers, it would be important to further explore the implications of protein kinase C activation by benzene.* Likewise, other growth factor pathways that could modify normal hematopoiesis need to be investigated in relation to benzene's effects.

Studies of benzene metabolism. The study of benzene metabolism over the past 40 years has identified a large array of metabolites (Snyder et al. 1992). It is not yet clear that all possible metabolites have been observed or identified. Metabolites that have been identified or for which there is good evidence include benzene oxide and its tautomer, oxepin; phenol; hydroquinone; catechol; benzene dihydrodiol; 1,2,4-trihydroxybenzene, the premercapturic acid of benzene; muconaldehyde; and the glucuronides and sulfates of the phenolic metabolites. It is postulated that *p*-, and *o*-benzoquinone may be terminal-reactive metabolites of hydroquinone and catechol, respectively. It is likely that a related quinone derivative is derived from 1,2,4-trihydroxybenzene. Several metabolites of muconaldehyde, reflective of the reduction or oxidation of each of the carbonyl functions, have been identified. If the dihydrodiol undergoes

further epoxidation, the resulting diol epoxide, which has produced lung tumors in the BLU:Ha newborn mouse assay (Busby et al. 1990), may be an important toxic metabolite. It may also give rise to a corresponding tetrol. *It should be determined whether the diol epoxide is formed.*

It is likely that benzene toxicity, and perhaps leukemogenicity, is caused by an array of metabolites rather than a single metabolite (Eastmond et al. 1987, Goldstein 1989, Guy et al. 1990, 1991).

- *Studies are needed to characterize the mixture of metabolites that are delivered from the liver to the bone marrow. It should be determined whether the mixture of metabolites can be altered and whether changing the mixture alters preselected toxic endpoints.*
- *Evaluation of the mixture of metabolites must include studies of the mechanisms by which metabolites leave the liver and enter the bone marrow.* For example, if ethereal sulfates are transport mechanisms, then how are they taken up into bone marrow cells? Are they hydrolyzed to release proximal toxic agents?
- *Investigation of the fate of benzene metabolites that enter the bone marrow is needed.* The marrow contains cytochrome P450, prostaglandin H synthetase, myeloperoxidase, and cytochrome *b-245* (respiratory burst enzyme), each of which may play a role in subsequent metabolism of benzene leading to ultimate toxic metabolites. These may inhibit cellular functions or act as reactive metabolites leading to covalent binding to cellular macromolecules.

In the last several years, several studies (Henderson et al. 1989, Sabourin et al. 1989, 1992.) have evaluated dose-response relationships for formation of benzene metabolites in various tissues of rats, mice, and monkeys. Although these data are useful for the development of physiologically based pharmacokinetic models, dose-response relationships for benzene metabolites and the mix of metabolites have not been well characterized in bone marrow.

- *Particular emphasis needs to be placed on the relationship between metabolite concentration and cell damage.*

DNA adduct studies. Reactive metabolites of benzene are known to react with glutathione, cellular protein, microsomal protein, sulfhydryl

groups of guanosine 5'-triphosphate GTP-tubulin, sulfhydryl groups of DNA polymerase γ , and nuclear and mitochondrial DNA (Snyder et al. 1992). A wide variety of cell types in the bone marrow have been shown to be affected by benzene or its metabolites or both. These include stem, progenitor, and other developing cells as well as cells of the hematopoietic microenvironment. Benzene and its metabolites have also been found to alter the production and effectiveness of interleukins and other growth factors in bone marrow.

- *Studies are needed to determine whether characterized DNA adducts for benzene metabolites are formed in vivo.* Benzene may increase oxidative damage in the form of 8-hydroxy 2'-deoxyguanosine (Kolachana et al. 1993), and benzene metabolites may possibly form protein cross-links that could interfere with normal cell regulation. The high reactivity of several benzene metabolites suggests that DNA adducts should readily form after benzene administration to rodents and be detectable by the ^{32}P -postlabeling assay of Reddy and Randerath (1986). Such adducts can be produced readily in vitro (Latriano et al. 1989, Low et al. 1991). However, demonstration in vivo has been elusive. Mazzulo and associates (1989) have seen adducts in vivo, but Low and colleagues (1991), in an extensive series of acute, subchronic and chronic oral benzene exposure studies, were unable to detect adducts in any of the likely target tissues.

Cell biology studies. Leukemia appears to be a multifactorial process that involves several steps in its development and cannot be described by a simple initiation-promotion paradigm. Acute myelogenous leukemia, the type of leukemia most often observed to result from exposure to benzene, may also follow treatment with antineoplastic therapeutic agents. Often patients go through a stage of therapy-related myelodysplastic syndrome (LeBeau and Larson, 1991) and about half of these will evolve to therapy-related acute myelogenous leukemia. Aberrations involving chromosomes 5 and 7 are commonly seen in patients with therapy-related myelodysplastic syndrome. In a group of such patients studied at the University of Chicago, 97% demonstrated chromosome aberrations. Of these 87% have abnormalities of chromosomes 5 or 7. In spontaneous acute myelogenous leukemia

with cytogenetic abnormalities, loss of chromosome 6 was observed in 9% of patients; loss of chromosome 5 was observed in 6% of patients (Le Beau and Larson, 1991). It therefore seems reasonable to assume that cytogenetic abnormalities, particularly breaks and nondisjunctional events, play a role in leukemia secondary to chemical exposure. The problem is that many agents that are toxic to bone marrow cause cytogenetic abnormalities; however, only some may cause leukemia and these have not been identified.

The connecting link could involve enhanced mitogenesis in selected populations of hematopoietic stem and progenitor cells that are targets of transformation. These are tightly controlled with respect to proliferation, and cytokine growth response is allowed in these cells by leukemogens. There are several recommendations for research in cell biology that would contribute to understanding of the biological basis for benzene-induced leukemia and lead to more rational risk assessment approaches:

- *Research is needed to understand species differences in stem cell regulation following exposure to benzene metabolites alone or in combination;*
- *The role of benzene-induced changes in cytokines and growth factor responses needs to be studied in relation to cell type and mitogenesis;*
- *Specific linkages of the steps in benzene-induced leukemia transformation need to be established. This will require analysis of different genes in mice and humans to clarify why humans get acute myelogenous leukemia but mice do not.*

GENETIC TOXICOLOGY

A potential initiating mechanism for benzene-induced leukemia in humans involves the production of a mutation in progenitor cells of the bone marrow. Cytogenetic investigations of leukemias have shown chromosomal aberrations. Clinically advanced leukemias generally have more karyotypic abnormalities than do cells in early disease stages.

Cytogenetic changes occurring early in the disease may be important in converting normal cells to the malignant state, but even a specific

chromosome change seen in all of a patient's malignant cells could have been produced by a submicroscopic lesion in a stem cell, with the initial cytogenetic change contributing to increased malignant potential (Nowell 1992). Changes at the chromosomal level are a major mechanism by which genes can be activated, altered, or inactivated to their malignant potential (Nowell 1992). Chromosome changes in leukemia include reciprocal translocations, gains or losses of whole chromosomes or pieces, and amplification of parts of chromosomes.

Approximately 50% of patients with acute nonlymphocytic leukemia (ANLL) have chromosomal abnormalities in bone marrow at the time of diagnosis. Translocations are the most common abnormalities (Heim and Mitelman 1992). In one case, t(8;21) (q22;q22), an altered gene product has been identified; in another, t(15;17) (q22;q11-12), a structurally altered receptor has been identified (de The et al. 1990, Miyoshi et al. 1991). Several other translocations have also been found, but altered genes have not yet been identified. Translocations may cause cell transformation by removing tumor suppressor genes or forming hybrid oncogenes.

In Vivo Animal Studies

Acute administration of benzene to rats by inhalation (100 or 1000 ppm for 6 hours) demonstrated a significant increase in marrow cells with chromosome aberrations (Styles and Richardson 1984). Studies in mice also demonstrate the clastogenic effects of benzene. Meyne and Legator (1980) showed that oral or intraperitoneal dosing of CD-1 mice with benzene caused an increased frequency of micronuclei with a higher frequency of micronuclei in males. Hite and associates (1980) also reported increased micronuclei in benzene-exposed mice.

In vivo assays of benzene and metabolites for genotoxicity have been limited primarily to cytogenetic evaluations. Hydroquinone has been shown to cause chromosomal aberrations in marrow cells of mice (Xu and Adler 1990), to increase aneuploidy (Parchieratti et al. 1991), and to induce micronuclei of bone marrow erythrocytes (Alder et al. 1989). Angelosanto et al. (1990) showed that acute, subchronic, and chronic oral administration of benzene to Fischer-344 or Sprague-Dawley rats produces dose-related

micronuclei formation in the Zymbal gland. In one study (Anwar et al. 1989), chromosomal aberrations were detected in mice receiving benzene at doses as low as 40 ppb for 6 weeks.

Tice and associates (1989) have demonstrated that phenobarbital increases both sister chromatid exchange (SCE) and chromosomal aberrations in benzene-exposed male mice, suggesting that metabolites are responsible for the effect.

In Vitro Studies

Many mutagenicity studies have been carried out with benzene and related metabolites. In most of the assays, benzene itself has been inactive. In one Ames assay (Glatt et al. 1989), a weak mutagenic response for benzene was seen in strain TA 1535. In the mouse lymphoma assay benzene and phenol were found to be mutagenic in the presence, but not in the absence of an S9 fraction; hydroquinone, catechol, benzenethiol, and benzoquinone were active in the presence of S9; and muconaldehyde was active in the absence of S9. In this experiment DNA adducts as measured by ³²P-postlabeling were not seen even at mutagenic levels; however, benzene in the presence of S9 produced dose-related increases in DNA strand breaks (Low et al. 1991).

Several in vitro cytogenetics tests have been positive for benzene (Palitti et al. 1985). These used protocols that took account of mitotic delay.

Assays for SCE induction in cultured human lymphocytes with S9 have shown benzene to increase SCE; reduced glutathione prevents the effect (Morimoto 1983). Erexson and colleagues (1985) showed that benzene, phenol, catechol, benzenetriol, hydroquinone, and benzoquinone induced SCE and inhibited cell cycle kinetics. Catechol was the most potent agent.

In vitro micronucleus assays in cultured human lymphocytes by Yager and associates (1990) showed that phenol, catechol, benzoquinone, and hydroquinone increased micronuclei formation, with hydroquinone having the most potency. Micronuclei were characterized as containing whole chromosomes, which indicates potential to cause aneuploidy.

Studies of Gene Alterations There is evidence in some types of cancer that mutations in the p53 suppressor gene may have caused predisposition to carcinogenesis (Vahakangas et al.

1992). Another type of injury leading to chromosome damage has been associated with promyelocytic leukemia (de The et al. 1991). Here, alteration of the gene for the retinoic acid receptor has led to the leukemia. Also, Taylor and associates (1992) have reported that leukemias with *ras* mutations are more likely to be associated with chemical exposure.

Research Recommendations. It is possible that benzene exerts a critical genetic effect in the leukemogenic process at the chromosomal level; however, further work is necessary to demonstrate the involvement of a genetic event in disease initiation.

- *Research to show which if any of the many chromosomal abnormalities associated with leukemia are produced by benzene exposure.* This may be possible with rodent studies.
- *In vitro and in vivo studies using the new whole-chromosome painting technique to locate specific chromosomes and determine frequency of translocations and chromosome loss that may occur after exposure to benzene and benzene metabolites.*
- *Studies to identify changes in proteins, receptors or cellular activities that result from chromosome damage.*
- *Development of an animal model of leukemia in which a target organ is shown to develop the same genetic lesion after exposure to benzene.* The rodent Zymbal gland may be a target organ that can be shown to develop such a lesion.
- *Identification of the benzene metabolites producing the critical cytogenetic effects, and an elucidation of the interplay between benzene metabolites to help clarify mechanisms of cell specificity and species differences.*
- *Generation of dose-response relationships for cytogenetic changes, if sufficient sensitivity can be achieved.*
- *Investigations into the mechanisms of aneuploidy by studying effects of benzene on centromeric-kinetochore proteins as well as microtubular protein.*
- *Investigations to determine whether benzene treatment in appropriate models results in alterations in p53 suppressor gene activity.*
- *Studies to determine whether benzene treatment in animal models causes changes in the*

expression of mRNA, proteins or functional measurements of specific oncogenes.

CARCINOGENIC RISK ASSESSMENT FOR BENZENE

Occupational exposure to benzene has been shown to increase the risk of leukemia and lymphatic and hematopoietic cancer in workers involved in the manufacture of rubber products (Infante et al. 1977; Rinsky et al. 1981, 1987; and Wong 1983). Benzene also induces solid tumors, notably Zymbal gland carcinomas, in rats and mice following both oral and inhalation exposure (Maltoni and Scarnato 1979, Maltoni et al. 1983, Huff et al. 1989). On the basis of these results, the International Agency for Research on Cancer has identified benzene as an established human carcinogen.

These same data have provided a basis for estimating the level of risk from human exposure to benzene. Because of the availability of dose-response data from cohort mortality studies, emphasis has been placed on the use of epidemiologic data in quantitative risk assessment (Voytek and Thorslund 1991). Pharmacokinetic data on metabolic production have also been used in risk assessment (Bailar and Hoel 1989; Belisles and Totman 1991; Cox and Ricci 1993).

Quantitative Risk Assessment

The EPA (U.S. Environmental Protection Agency 1979) used epidemiologic data reported by Aksoy (1977), Infante and associates (1977), and Ott and associates (1978) to derive a unit (lifetime) risk of 0.024 ppm^{-1} based on an essentially linear one-hit model. This was later modified to 0.026 ppm^{-1} on the basis of new epidemiologic data reported by Rinsky and associates (1981) and Wong (1983) (U.S. Environmental Protection Agency 1985). (In both cases, an average risk across studies was used.) This transforms to a unit risk of $0.28 \text{ (mg/kg/day)}^{-1}$ for oral exposure. These estimates were confirmed in a later review by the CRAVE Work Group within the EPA (U.S. Environmental Protection Agency 1989).

Crump and Allen (1984) reviewed and updated the estimates of worker exposure in the cohort assembled by Rinsky and associates (1981). Combining the cohort data of Rinsky and

associates (1981) with the cohort of Ott and associates (1978), and using a linear model, Crump and Allen (1984) obtained unit risks of between 0.0012 and 0.0095 ppm^{-1} depending on the exposure modeling procedure used. Thorslund and colleagues (1988) conducted a similar analysis based on a special case of the two-stage clonal expansion model of carcinogenesis that is linear-quadratic. In the latter analysis, emphasis was placed on modeling absolute risk (i.e., age-specific incidence) because the relative risk of benzene-induced leukemia does not appear to be constant over time. This analysis, based on the Rinsky and associates (1981) cohort only, provided a unit risk of about 0.0078 ppm^{-1} .

Belisles and Totman (1989) used a Michaelis-Menton model to describe the formation of benzene metabolites in animals. Using the linearized multistage model to describe the incidence of Zymbal gland tumors in rodents and allometric procedures to scale these results to humans, they estimated the lifetime risk for occupational exposure (40 hours per week for 45 years) to be 0.001 ppm^{-1} . This estimate is comparable to risk estimates for leukemia based on epidemiologic data.

Bailar and Hoel (1989) used pharmacokinetic information to estimate cancer risks based on metabolite formation. Cox and Ricci (1993) conducted a similar analysis using the physiologically based pharmacokinetic model for benzene metabolism developed by Travis and associates (1990). In both analyses, the dose-response relationship appeared to be nonlinear, leading to a much lower value of 10^{-7} ppm^{-1} for the unit risk of benzene effects under continuous lifetime exposure. However, Crump et al (1993) found that the Cox and Ricci method applied to other endpoints yielded much higher unit risks. Ricci and Cox (1993) recently used a bootstrap method to evaluate the uncertainty in this estimate under the essentially cubic model developed for benzene metabolites; even allowing for experimental error, this analysis leads to markedly lower risks than in previous analyses.

Recent Developments

Further data are now available for cancer risk assessment. Rinsky and associates (1987) updated the mortality experience of their cohort, taking into account another 123 deaths since their original

report on the pliofilm workers in 1981. Paustenbach and colleagues (1992) have completed a detailed review of the historical exposure of workers in the cohort, reporting exposures higher or lower than the exposures estimated by Crump and Allen (1984), depending on job category, but notably higher than the exposures estimated by Rinsky and associates (1987). Paxton and colleagues (1992) used these updated exposure data and a proportional hazards model based on cumulative exposure to benzene to obtain an estimate of risk of about 4×10^{-4} ppm⁻¹ based on occupational exposure (40 hours per week for 45 years). This is less than the corresponding risk of 5×10^{-3} ppm⁻¹ based on the report by Rinsky and associates (1987) (see also Brett et al. 1989). This reduction was attributed to the higher exposure estimates reported by Paustenbach and colleagues (1992) for the pliofilm workers as compared with the estimates of Rinsky and associates (1987).

Biological Markers

Johnson and Lucier (1992) recently discussed the use of muconic acid, a metabolite of benzene, as a biomarker of benzene exposure. Muconic acid has been detected in the urine of workers at levels suggesting occupational exposures in excess of 5 ppm. The presence of muconic acid in urine samples from the general population also suggests exposure in excess of 1 ppm.

Taken at face value, this latter observation suggests that environmental exposures could account for a significant proportion of the leukemia cases in the population at large. To resolve this, information on interindividual variation in the metabolism of benzene is required as well as on the specificity of muconic acid as a marker of benzene exposure. Depending on these results, a detailed systematic study of environmental exposure to benzene may be warranted.

DOSE-RESPONSE MODELING

Numerous research topics related to improving the assessment of the relationship between exposure and response can be identified. These include the development of new models, collection of data to clarify mechanisms, and the combination and use of existing models to improve current risk

estimates. What follows is a brief description of these issues.

A major gap in the understanding of benzene-induced toxicity is our knowledge of the role, if any, of bone marrow toxicity and aplastic anemia in the development and progression of leukemia in humans. Laboratory research into clarifying this relationship is discussed in another part of this document. However, dose-response modeling for benzene-induced human leukemia does not need to wait for further research. Existing models of cellular differentiation and maturation in the human hematopoietic system should be used to ask questions about what could be the effect of various assumed mechanisms on the resulting distribution of mature blood cells in humans. This simulation could be compared with existing hematoxic data on reduction in blood erythrocytes, leukocytes, and thrombocytes as a first attempt to describe reasonable mechanisms. This research has the advantage of being inexpensive. In addition, it could possibly aid in the design of mechanistic laboratory studies.

Development of a comprehensive human dose-response model for benzene-induced leukemia should be started. The whole array of models (exposure, dose delivery, gene expression, cell and tissue response, and host survival) should be addressed. Because of gaps in our data base for dose response for specific endpoints, many of the dose-response relationships will be intractable. Nevertheless, the extent to which a model can be developed on the basis of current understanding of benzene toxicity and current data will go a long way toward defining further research needs for risk estimation. This research has the distinct advantage of being inexpensive and can be done in a timely fashion.

Physiologically based pharmacokinetic models for benzene have been published. These models need to be expanded to include physiologic distribution to and metabolism in the bone marrow. They also need to be extended to include a larger array of metabolites, especially those that have potential utility as biomarkers in humans (such as muconaldehyde). This will be useful for extrapolation of toxic effects across species. The fate of these metabolites should be modeled and should include transport of metabolites from one tissue to another. Particularly important are metabolites of benzene that enter the bone marrow. The potential effect of these metabolites

on cellular function should be quantified. Although there may be little or no data that will allow for the quantification to be completed, this research should help to identify data gaps. In addition, hypothetical dose-response models can be used to determine the potential of further data for modifying risk estimates and reducing uncertainty. This research is also inexpensive and is likely to be accomplished in a short time frame.

Research Recommendations

- *A comparison should be made between the toxic effects following repeated, short-term, high-level exposures and those resulting from chronic low-level exposures.*

Bioassay experiments defined to address this issue should not necessarily be lifetime studies for carcinogenicity. The studies done by Cronkite and associates (1989) should be expanded to include additional doses, especially in the low-dose range, and measurement of metabolite levels in the various tissues. This research will increase understanding of the effect of dose rate on the toxicity due to benzene.

- *Dose rate effects must be understood if extrapolation across species is to be attempted.*

The relationship between age of exposure, magnitude of exposure, and rate of exposure on tissue response should be clarified to resolve a portion of the controversy on proper estimates of exposure in the occupational populations that have been studied for benzene-induced leukemia.

PRIORITIZATION OF RESEARCH RECOMMENDATIONS

The descriptions presented in this background document on research recommendations to decrease uncertainty in risk assessment for benzene contain many recommendations for research. Some of these recommendations are long-term and some are short-term, and some have greater chances of success than others. Although fulfillment of each of the recommendations would strengthen the scientific foundation on which benzene risk assessments are placed, the following would be especially helpful in decreasing uncertainty.

Exposure

- *In the area of exposure, the question of microenvironments that create substantial opportunities for benzene exposure must be better understood.*

Cancer

Cancer is the health effect that is of greatest concern following environmental exposures to benzene, and there are a number of questions about it that must be answered.

- *First, does benzene cause solid tumors or hematological neoplasms other than acute nonlymphocytic leukemia in humans? This question might best be answered by continued follow-up of existing worker cohorts or by cohorts now being established in China. In addition, attempts should be made to use valid metaanalytic approaches for use in generating more sound risk assessments.*
- *Second, the tools of molecular biology offer the opportunity to develop sensitive and specific markers of exposure and to identify susceptible populations on the basis of polymorphisms in benzene-metabolizing enzymes and inherent sensitivities or existing damage of blood cell types. Well-designed molecular epidemiologic studies, using sound epidemiologic and laboratory approaches, may permit better characterization of benzene's cancer effects and also help to clarify the shape of the dose-response curve in the low-dose region.*
- *Third, considerable uncertainty in benzene risk assessments is created by the lack of a suitable animal model. Development of such a model, although extraordinarily difficult, would allow for the design of much-needed mechanistic studies.*

Dose-Response Models

The development of acceptable mechanistically based or biologically based dose-response models could become the cornerstone of more reliable risk assessments for benzene. Obviously, such models require a reasonable, not necessarily complete, understanding of mechanisms by which benzene causes leukemia or perhaps other cancers.

Mechanisms

Although we have listed several research needs related to mechanisms, the following warrant special consideration.

- *What is the mechanism by which combinations of benzene metabolites produce greater than additive cytogenetic and hematopoietic effects?*
- *What are the critical target genes or cells for benzene's effects?*
- *Is pancytopenia or aplastic anemia an essential step in benzene-induced leukemia?*
- *What is the role of benzene-induced changes in cytokines and growth factor pathways relevant to leukemia?*

Chromosomal Changes and Leukemia

Numerous studies in the literature discuss the cytogenetic effects of benzene in in vivo and in vitro systems. Yet we still do not have sufficient understanding of the link between specific chromosomal changes and leukemia, so research is recommended in this area. Such research may be aided by techniques such as whole-chromosome painting and studies on the mechanism of benzene-induced aneuploidy.

- *The development of biologically based dose-response models requires the valid translation of biology into mathematics. There is increasing recognition that the development of these models helps to pinpoint research needs to address the greatest sources of uncertainty. For this reason, we strongly recommend the development of a comprehensive dose-response model for benzene-induced leukemia. This model could be modified as new biological or mechanistic data are obtained, leading to a reduction in uncertainty with each modification.*
- *We also recommend the development of a model to determine the probability of a link between aplastic anemia and leukemia. This model could use existing information on cellular differentiation and maturation in the human hematopoietic system.*

In summary, it is clear that research aimed at reducing uncertainty in benzene risk assessments will be well served by effective interactions between epidemiologists, mathematicians, molecular biologists, and toxicologists, so we recommend the development of multidisciplinary

teams to address many of the high-priority research projects presented in this document.

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Research Priorities to Reduce Uncertainties in Risk Assessment for 1,3-Butadiene

INTRODUCTION

1,3-Butadiene (BD) is a four-carbon gaseous combustion product and a monomer that is used in synthetic rubber production. Because of its two double bonds, 1,3-butadiene is metabolized to the monoepoxide, 1,2-epoxy-3-butene (BDO), and to a lesser extent the diepoxide, 1,2,3,4-diepoxbutane (BDO₂). 1,3-Butadiene is therefore an indirect genotoxic alkylating agent for DNA with the potential of being a cross-linking agent for DNA and protein.

1,3-Butadiene causes tumors in both mice and rats, and there is suggestive evidence that it causes cancer in humans exposed occupationally. In the classification system of the U.S. Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC), 1,3-butadiene is ranked as a probable human carcinogen.

A central issue in 1,3-butadiene health assessment is to confirm its carcinogenicity in humans and improve estimates of carcinogenic potency. There are at present no solid human dose-related cancer studies on which to base a potency estimate and it is unclear which laboratory animal species is most appropriate to use for human extrapolation. In animal studies, the potency for tumorigenesis is greater in mice than in rats, and there is a different spectrum of tumor types in the two species, so there are some tumors in the rat that are not induced in comparable doses in the mouse.

Some general issues with respect to 1,3-butadiene are the following:

- Can we obtain more decisive epidemiologic cancer-confirmation and potency data in humans?

- Can we learn enough about carcinogenic mechanisms to decide which animal species, rats or mice, if either, is a useful potency surrogate for humans?
- Can we understand enough about the variability in human metabolism to determine whether there are sensitive individuals who the standards need to protect?
- Is the reproductive and developmental toxicity of 1,3-butadiene an important issue?

A list of research recommendations considered to be of high priority can be found in the Prioritization of Research Recommendations section.

EXPOSURE ASSESSMENT

Information relating to the estimation of human and environmental exposures to 1,3-butadiene has been reviewed recently (U.S. Environmental Protection Agency 1985, 1989, 1992; Toxics Release Inventory 1990; California Air Resources Board 1991; International Agency for Research on Cancer 1992).

1,3-Butadiene is a flammable, colorless gas (boiling point -4.5°C) with a mildly pungent odor. In 1987, worldwide production of 1,3-butadiene constituted some 5.5×10^6 megagrams (= Mg, metric tons), mostly generated during ethylene production. Of this, 1.36×10^6 Mg was produced in the United States, which has 11 production facilities, all located in Texas or Louisiana.

Tobacco smoke is thought to be the primary source of 1,3-butadiene in indoor air. It is emitted primarily from sidestream smoke at a rate of about $400 \mu\text{g}$ of 1,3-butadiene per cigarette (California Air Resources Board 1991). Gasoline may contain small amounts of 1,3-butadiene, and its production, and handling may result in some releases. By far the largest source of 1,3-butadiene emissions, however, is incomplete fuel combustion, principally from on-road vehicles, but also from boats, trains, ships, aircraft, and stationary sources such as residential heating, boilers, turbines, and internal combustion engine-driven machinery (U.S. Environmental Protection Agency 1989, 1992; California Air Resources Board 1991).

Owing to its volatility and poor solubility in water, any releases of 1,3-butadiene not made directly to the atmosphere will quickly end up

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there. For the same reasons, neither aqueous phase atmospheric chemistry nor incorporation into clouds and rain are important determinants of 1,3-butadiene's fate (U.S. Environmental Protection Agency 1989, 1992; California Air Resources Board 1991). Removal from the atmosphere occurs principally from reaction with hydroxyl ($\cdot\text{OH}$) and nitrate ($\cdot\text{NO}_3$) radicals, and reaction with ozone (O_3) (California Air Resources Board 1991). The removal rates depend on temperature and the concentrations of the reagents, which in turn vary geographically, diurnally, seasonally, and between cloudy and sunny weather (California Air Resources Board 1991, U.S. Environmental Protection Agency 1992). The fastest reaction, with $\cdot\text{OH}$ radicals, dominates during the day, while reaction with $\cdot\text{NO}_3$ generally dominates at night. Under favorable conditions, the residence time of 1,3-butadiene (the time to reduce the initial concentration by a factor of $1/e$, i.e., to about 37% of the original amount) can be quite short, about 0.5 to 1 hour on a clear summer day. On a cloudy winter night, in contrast, a projected residence time may be thousands of hours (California Air Resources Board 1991, U.S. Environmental Protection Agency 1992). Under most conditions, however, a great deal of day-to-day carryover is not expected, and ambient 1,3-butadiene will usually be confined to a local radius of impact within the airshed in which it was emitted (California Air Resources Board 1991, U.S. Environmental Protection Agency 1992). Formaldehyde and acrolein ($\text{CH}_2=\text{CHCHO}$), the primary products of atmospheric breakdown of 1,3-butadiene are themselves pollutants of interest (see Aldehydes chapter). 1,3-Butadiene breakdown is a minor source of atmospheric formaldehyde but may be the principal source of acrolein (California Air Resources Board 1991, U.S. Environmental Protection Agency 1992).

Emissions

Emissions from industrial production and use arise from processing vents, equipment leaks, waste streams, storage, and accidental or emergency release. The 1990 Toxics Release Inventory lists 162 sources emitting a total of 2,284 Mg of 1,3-butadiene to the air and a further 3 Mg to land and 51 Mg to water disposal. Fourteen sources emitted more than 50 Mg, accounting together for more than 57% of the total. In addition to these

self-reported data on total annual releases, there have been estimates of process-specific and episodic releases, both nationally (U.S. Environmental Protection Agency 1987, 1989; Mullins 1990) and in California (California Air Resources Board 1991), based on analysis of process descriptions, equipment inventories, and reported plant capacities. The relatively rapid atmospheric degradation of 1,3-butadiene (see above) and the much larger total magnitude of emissions from vehicular exhaust (see below) suggest that industrial emissions may constitute fairly localized "hot spots" within a much broader overall pattern of human exposure.

The EPA has estimated 1990 emissions from on-road mobile sources as 27,972 Mg (U.S. Environmental Protection Agency 1992). Emissions from off-road mobile sources (including ships, trains, and aircraft) were not estimated but may also be considerable; the California Air Resources Board estimated that such emissions were 28% of all mobile source emissions in that state (California Air Resources Board 1991). If this ratio were to apply nationally (which it probably does not), one would estimate additional emissions of 10,800 Mg. Overall, then, these estimates indicate that the bulk of 1,3-butadiene emitted to the atmosphere arises from incomplete combustion of fuel, mostly from on-road vehicles. No national estimates of emissions from stationary sources burning fossil fuel seem to be available. California's estimate (California Air Resources Board 1991) is that 4% of its total 1,3-butadiene emissions arise from such sources, including residential heating.

The emissions of 1,3-butadiene from vehicular fuel-burning are estimated by using an empirically determined concentration of 1,3-butadiene in total organic exhaust gases (TOG). This value is multiplied by the rate of emissions of TOG for vehicles of different classes to get 1,3-butadiene emission per mile. The 1,3-butadiene emissions per mile are converted to total 1,3-butadiene emissions by applying data (or projections) of vehicle-miles traveled. (California Air Resources Board 1991, U.S. Environmental Protection Agency 1992). (Emissions from other fuel-burning are estimated in an analogous way.) 1,3-Butadiene emissions maintain rough proportionality to TOG, so steps to reduce such emissions will reduce 1,3-butadiene emissions to a similar degree. Emissions projections are complicated by the wide variety of

vehicles in use, by the use of different fuels (gasoline vs. diesel), and by different systems for catalytic treatment of exhaust. Moreover, projections of future emissions must address anticipated and potential changes in mileage and emissions standards, as well as a number of proposals for reformulation of fuels. Projections under development by the EPA Office of Mobile Sources (U.S. Environmental Protection Agency 1992) indicate that these factors will result in reductions of about 60% in emissions per mile by 2010. (Increases in vehicle-miles traveled, however, are projected partially to counterbalance this reduction, with total emissions dropping by about one-third by 2000, then beginning to rise again.)

Occupational Exposure

A National Institute for Occupational Safety and Health survey (NIOSH 1984, Fajen et al. 1990) estimates that some 65,000 American workers may potentially be exposed to 1,3-butadiene. Industrial hygiene data on 1,3-butadiene are not particularly good, especially before the 1980s, since most exposures were well below the Occupational Safety and Health Administration (OSHA) limit, samples were few, and sampling methodology was not rigorous. Several more recent studies, summarized by the International Agency for Research on Cancer (1992), show that most occupational exposures are below an 8-hour time-weighted-average (TWA) of 1 ppm. As a reactive gas, 1,3-butadiene is usually well confined in most industrial processes. Certain jobs that involve transferring 1,3-butadiene or otherwise interrupting its enclosure, such as railcar worker, tank truck operator, and maintenance worker, may have higher exposures, on the order of 2 to 20 ppm as 8-hour TWA and including peaks upward of 100 ppm.

Ambient Air Exposures

There has been relatively little investigation of ambient air levels of 1,3-butadiene and still less of its geographic and temporal variation. Scattered reports, collected by different methods using 8 different sampling times and conditions, generally show that urban air typically contains from 1 to 10 ppb (International Agency for Research on Cancer 1992, U.S. Environmental Protection Agency 1983), or roughly 1,000-fold lower than occupational levels. By far the best study was

conducted by the California Air Resources Board using its toxic monitoring network (1991). Twenty sampling locations throughout the state were chosen according to the EPA criteria for siting ambient air monitors, and from 12 to 24 24-hour samples were taken per site on randomly chosen days over a one-year period from July 1988 through June 1989. Estimated annual average concentrations over sites ranged from about 0.2 to 0.4 ppb, with the maximum detected concentration being 1.7 ppb. A population-weighted overall mean for the state's ambient air was 0.37 ppb. (The geographic mean was somewhat lower because 1,3-butadiene levels tended to be higher in more populated areas.) A second California Air Resources Board study, focusing on the South Coast Air Basin, detected an average of 1.6 ppb (ranging up to 17.7 ppb) in one-hour air samples taken under well-documented meteorologic conditions (California Air Resources Board 1991). These results, somewhat higher than the toxic monitoring network results, illustrate the importance of conditions modulating atmospheric breakdown rates, noted above. Reports from the Texas Air Control Board show much higher peak measurements compared to that of CARB. Peak levels for two different years near a production facility in Texas were of 1.7 ppm and 2.9 ppm over a period of a week (Texas Air Control Board 1989, 1990).

Indoor Air Exposures

1,3-Butadiene is found in sidestream cigarette smoke, and studies of smoky taverns found levels of about 2 to 8 ppb (while simultaneous outdoor levels were below 0.45 ppb). A California Air Resources Board study of homes, however, found generally low levels, often lower than outdoor ambient air (California Air Resources Board 1991).

Research Recommendations. To assess the scope and magnitude of human exposure, one needs to establish the air concentrations of 1,3-butadiene as they are encountered by people, as well as the numbers of people who suffer such encounters, and the frequency and duration of those encounters. Information on sources and emissions are important in identifying probable exposure pathways and in giving a general idea of their significance, but a full exposure assessment must proceed two steps further: first to characterize

characterize (through modeling or monitoring) the geographic and temporal patterns of air concentrations that result from the interplay between emission rates on the one hand and transport and destruction processes on the other and second to characterize the patterns of human encounter with these concentrations. The information on which to carry out these further steps is poorly developed in the case of 1,3-butadiene.

Sources. Emissions from the major industrial point sources could be better characterized, especially with respect to episodic emissions. Point sources too small to show up on the Toxics Release Inventory may nonetheless be locally (and collectively) important, yet there is virtually no information on them. Vehicle emissions have been projected on a relatively narrow base and would benefit from fuller characterization of variation among vehicle types. The impact of changes in emissions standards and fuel reformulation on emissions needs further clarification. Owing to the magnitude of mobile source emissions, small changes in these factors may have a major impact on the overall exposure assessment. National estimates of off-road mobile source emissions are needed. Emissions estimates for stationary source fuel-burning are based on few data; such emissions are potentially important to ambient air concentrations, especially since some (such as residential heating) may occur late at night during times of slow atmospheric destruction. The role of home heating and gas appliances in indoor air levels of 1,3-butadiene is unknown. Since cigarette smoke contains 1,3-butadiene, perhaps other combustion materials (aside from petroleum-based fuels) may be 1,3-butadiene sources.

The aim in this area is to better characterize the fraction of total ambient 1,3-butadiene attributable to mobile sources. Therefore, the research recommendations are the following:

- *Better characterization of emissions from on-road vehicles, especially for some classes of vehicles, and from off-road mobile sources (aircraft, trains, ships).*
- *More direct characterization of the effects of fuel reformulation and changing emissions standards on 1,3-butadiene in exhaust in order to better project future risk.*
- *Measurements of emissions factors for combustion of petroleum-based fuels other than*

in engines (e.g., in residential heating commercial boilers).

- *Measurements of 1,3-butadiene emissions from combustion of other fuels (incineration, coal, wood-burning).*
- *Collection of data for estimation of national emissions from off-road mobile sources (in addition to emissions factors).*

Fate and Transport. The reaction rate of ozone with 1,3-butadiene is not well characterized, and the fate of some of the initial reaction products is not known (California Air Resources Board 1991). To characterize the dependence of destruction processes on weather and the presence of reactive chemicals, better characterization of the dynamics of nitrate and ozone would be helpful. There are few empirical data on 1,3-butadiene levels as functions of variation in these factors, although theoretical grounds suggest potential major impact.

The overall aim of the research recommendations in this area is to relate emissions to ambient levels.

- *Better determination of reaction rate of 1,3-butadiene with tropospheric ozone.*
- *Collection of data on concentrations of hydroxyl and nitrate radicals and ozone as they vary geographically, daily, seasonally, and with weather conditions, for use in models of 1,3-butadiene fate and transport.*
- *Fate and transport modeling of 1,3-butadiene incorporating the dynamics of emissions and destructive processes to characterize geographic and temporal variation in resulting ambient air concentrations.*

Ambient Air Exposures. There are few data on ambient air levels, and those that exist are of little help in characterizing geographic and temporal variation. Rates of atmospheric destruction suggest that ambient exposures will generally be local and immediate, but there is little quantitation. The extent to which major point sources create local areas of elevated ambient concentrations is not known. (California Air Resources Board [1991]) has announced its intention to conduct a study on this matter). In sum, the geographic inhomogeneity of emissions is expected to combine with the rapid atmospheric destruction of 1,3-butadiene (the rates of which vary with season, weather, time of day, and levels of other pollutants) to produce a pattern of higher and lower ambient air concentrations in

different locations, a pattern that will shift over time. The few available monitoring data show less patchiness than might be expected, but the data are sparse enough that characterizing such patterns would be difficult. In principle, modeling could help to answer these questions; there are data on emissions, on the forces affecting fate and transport, on typical meteorologic conditions, and on the geographic and temporal variation of hydroxyl and nitrate radicals and ozone, and a model that brings these factors together could be helpful in predicting the degree of fluctuation in ambient levels. Initial attempts at such modeling (U.S. Environmental Protection Agency 1992) are encouraging, but do not answer key questions.

In order to characterize the patterns and levels of exposures to humans, the following research recommendations are made.

- *Investigate the extent of elevated ambient concentrations around major point sources, using modeling and monitoring.*
- *Use ambient air monitoring to discover the extent of temporal and geographic variation in ambient air concentrations, including levels in key microenvironments such as within automobiles, in garages, and in tunnels.*
- *Develop, validate, and use biomarkers of exposure to assess total exposures as experienced by the general population.*

METABOLISM, BIOCHEMISTRY, AND TOXICOKINETICS

The chemical structure of 1,3-butadiene suggests that it is metabolized via cytochrome P-450 enzymes to mono- or diepoxides followed by hydrolysis to hydroxy compounds and/or conjugation with glutathione. Because it is a small compound, one would also expect that the nonreactive forms of the metabolites may enter the metabolic pathways for 4-carbon units or be oxidized to CO₂ for energy. The current state of knowledge on the metabolism of 1,3-butadiene is summarized in Figure 1.

In Vitro Studies

Malvoisin et al. (1979) first reported that 1,2-epoxy-3-butene (1,3-butadiene monoepoxide, BDO) is the primary 1,3-butadiene metabolite

formed by rat liver microsomal preparations in the presence of an NADPH-generating system. Later they reported that the cytochrome P-450-linked mixed function oxidases of rat liver microsomes would also convert BDO to the diepoxide, 1,2,3,4-diepoxybutane, (BDO₂) and that the epoxide hydrolases of the microsomes converted the BDO to the butene diol compound (1,2-dihydroxy-3-butene) (Malvoisin and Roberfroid 1982). Bolt et al. (1983) confirmed the formation of BDO from 1,3-butadiene in rat liver microsomes and showed that the formation of BDO was inhibited by the monooxygenase inhibitor SKF 525 A and was enhanced by inhibitors of the epoxide hydrolase (1,1,1-trichloropropene oxide). The investigators also found that addition of reduced glutathione (GSH) and liver cytosol reduced the amount of BDO in their incubation, suggesting that BDO could react with GSH in the presence of glutathione transferase (GT) to form conjugates.

Species differences in the rate of formation of BDO from 1,3-butadiene were reported by Schmidt and Loeser (1986) using both liver and lung microsomal preparations. In the liver, the rate of formation of BDO (nmol/min/g) was highest in microsomes from the mouse and was lower in rats, humans, and rhesus monkeys. The ratio of the rate in the mouse to that in the monkey was 7:1. In the microsomal preparations from lungs, only the rodents produced any BDO. In the mouse, the formation of BDO in lung preparations was comparable to that in liver, even though the mouse lung tissue has only one-thirtieth the total monooxygenase activity of the mouse liver tissue. A limitation of the study was the use of samples from only one human subject.

Csanády and associates (1992) compared the metabolism of 1,3-butadiene and BDO in liver and lung tissues from humans (12 individuals), rats, and mice. Maximum rates for 1,3-butadiene oxidation were highest for mouse liver microsomes (2.6 nmol/mg protein/min) compared with humans (1.2 nmol/mg/min) and rats (0.6 nmol/mg/min). The maximum rate of 1,3-butadiene oxidation in mouse lung was similar to that in mouse liver but was more than 10 times higher than in human or rat lung microsomes. The major P-450 enzyme responsible for the 1,3-butadiene oxidation was P-450 2E1. Only mouse liver microsomes demonstrated detectable rates of metabolism of BDO to BDO₂. The hydrolysis of BDO to 1,2-dihydroxy-3-butene was highest in

human liver microsomes and was two-fold higher than for rat or mouse liver microsomes. The maximum rate of conjugation of BDO with GSH was highest in mouse liver cytosol (500 nmol/mg protein/min) compared with human (45 nmol/mg/min) or rat (241 nmol/mg/min) liver cytosol. The authors reported overall activation:detoxication ratios for the livers as 12.5:1 in mice, 1.3:1 in rats and 4.4:1 in humans (Csanády et al. 1993).

Studies by Elfarra and associates (1991) indicate that the oxidation of 1,3-butadiene by either the mouse liver microsome system or the H_2O_2 /chloroperoxidase (from the fungus *Caldariomyces fumago*) system produces both BDO and crotonaldehyde. The investigators suggest that the intermediates formed in the monooxygenase epoxidation can either undergo ring closure to form BDO or, through a hydrogen shift, form 3-butenal, which tautomerizes to form crotonaldehyde. Two other proposed metabolites, methyl vinyl ketone and 2,5-dihydrofuran, were not found.

In Vivo Studies

BDO has been confirmed as a major metabolite of 1,3-butadiene in vivo in Sprague-Dawley rats (Bolt et al. 1983, Filser and Bolt 1984).

Work by Bond and associates (1986, 1987), Dahl and associates (1990, 1991), and Sabourin and associates (1992) illustrates species differences in the metabolism of 1,3-butadiene. In inhalation exposures to low levels (≈ 10 ppm) of 1,3-butadiene, B6C3F₁ mice absorbed 20% of the inhaled ¹⁴C-1,3-butadiene while Sprague-Dawley rats and cynomolgus monkeys absorbed 4% or 3% (Dahl et al. 1991). Of what was retained, the excretion patterns for the 1,3-butadiene-derived ¹⁴C were similar in the two rodent species, but the monkeys exhaled half of the retained ¹⁴C-1,3-butadiene equivalents as carbon dioxide (CO₂) while the rodents exhaled only 12% (rats) or 4% (mice) as CO₂. Tentative identification of blood metabolites indicated that all species had higher levels of BDO than BDO₂ in the blood.

Two major urinary metabolites of 1,3-butadiene have been identified in mice, rats, and monkeys by gas chromatography/mass spectrometry (GC/MS) techniques (Sabourin et al. 1992). The metabolites were the mercapturic acids formed from conjugation of GSH with BDO, 1-hydroxy-2-(N-

acetylcysteinyl)-3-butene (called II in Fig. 1) and with 1,2-dihydroxy-3-butene (1,2-dihydroxy-4-(N-acetylcysteinyl)-butane), (called I in Fig. 1). The corresponding glutathione conjugates have been detected in the bile of rats treated with 1,3-butadiene (Sharer and Elfarra 1992). The ratio of I to I + II in urine varied linearly with epoxide hydrolase activity in the livers of the different species and was highest in the monkey and lowest in the mouse. These data indicate that the mouse is not able to hydrolyze BDO as efficiently as the rat or monkey and thus excretes more 1,3-butadiene in the form of BDO conjugates than the other species. The monkey, on the other hand, appears to be efficient at hydrolyzing BDO to 1,2-dihydroxy-3-butene and thus excretes mainly the mercapturic acid formed from its GSH conjugate. Unpublished data indicate that only metabolite I, and not metabolite II, can be detected in the urine of workers occupationally exposed to low (< 3 ppm) levels of 1,3-butadiene (Bechtold WE, Strunk MR, Ward JR, Henderson RF, submitted). The data support the studies done in vitro that indicate the mouse has a lesser capacity than humans to hydrolyze BDO but a greater capacity to form glutathione conjugates with BDO (Csanády et al. 1992).

The differences in carcinogenic sensitivity of rats and mice to inhaled 1,3-butadiene suggest that toxicokinetics may contribute, in part, to the observed species differences. However, tissue concentrations of BDO, predicted in rats and mice from a physiologically based pharmacokinetic model of uptake, tissue distribution, and metabolism of 1,3-butadiene did not correlate with differences in tumor incidence in these species (Kohn and Melnick, 1993).

In the closed-chamber studies of Bolt and colleagues (Bolt et al. 1984, Kreiling et al. 1986), in which the decline in 1,3-butadiene concentration in the chamber is due to uptake and metabolism, the disappearance of 1,3-butadiene was nonlinear at exposure concentrations greater than 1,000 ppm, with metabolic saturation occurring at 1,3-butadiene concentrations greater than 1,500 ppm in both rats and mice. Two parameters, maximal metabolic elimination rate and clearance, suggest that significant species differences exist in 1,3-butadiene disposition. For example, the maximum metabolic elimination rate in mice was approximately twice that in rats. Further, clearance of 1,3-butadiene was nearly two-fold greater in

mice than in rats. In closed-chamber studies in rats (Filsler and Bolt 1984, Kreiling et al. 1987), saturation kinetics of BDO metabolism were not observed up to 5,000 ppm BDO. In mice, saturation of BDO metabolism occurred at about 500 ppm. Furthermore, in the linear range (< 500 ppm), the steady-state concentration of BDO in mice was approximately 10 times that in rats. Rats are able to eliminate BDO faster than mice, with a maximum elimination rate that is 8 times greater than that observed in mice. Thus, mice appear to have a relatively lower detoxication capacity than rats. These data are consistent with *in vitro* studies of Csanády and associates (1992) using rat and mouse hepatic microsomes and cytosol and with studies by Laib and associates (1988).

Studies using dynamic, nose-only exposures of B6C3F₁ mice, Sprague-Dawley rats, and monkeys to ¹⁴C-1,3-butadiene support the data from the closed-chamber studies (Bond et al. 1986, 1987; Dahl et al. 1990, 1991; Sabourin et al. 1992). Mice and rats were found to absorb a greater fraction of the inhaled 1,3-butadiene than monkeys. Mice had higher levels of 1,3-butadiene metabolites than rats and monkeys. Urinary metabolites indicated that mice removed BDO by conjugation with GSH (excreted as metabolite II). The distribution of ¹⁴C-1,3-butadiene-derived radioactivity was examined in tissues of rats and mice (Bond et al. 1987). Few differences were observed. As might be expected, fat was a major tissue depot.

In summary, the available toxicokinetic evidence suggests that mice possess a greater capacity to metabolize 1,3-butadiene to BDO than do rats or monkeys, and a lesser capacity to hydrolyze BDO.

Summary of Current Knowledge

Our current knowledge of the metabolism of 1,3-butadiene is indicated in Figure 1. Much of the information is derived from *in vitro* studies, but the studies *in vivo* support the findings *in vitro*. The primary metabolite, BDO, has been detected, either *in vitro* or *in vivo*, in all mammalian species. This is significant because BDO is mutagenic and carcinogenic. The mouse forms BDO efficiently and conjugates it with GSH. In comparing mice, rats, and primates, there is an increasing fraction of the BDO that is rapidly hydrolyzed to 1,2-dihydroxy-3-butene and excreted by conjugation of GSH with that compound.

It appears from studies on the rates of metabolism of 1,3-butadiene and BDO that B6C3F₁ mice may accumulate more BDO in blood and tissues than rats or monkeys. Data confirming this are still tentative for the most part, although high levels of BDO have been reported in 1,3-butadiene-exposed mice. Further studies are required to clarify these points.

Research Recommendations. The major unresolved issue is how humans metabolize 1,3-butadiene compared with rodents. More information is required on the rates of metabolism of 1,3-butadiene, BDO, and BDO₂ *in vitro* and *in vivo*, in humans as well as rodents. The information in Figure 1 indicates that there are uncertainties as to whether many of the metabolites formed *in vitro* also occur *in vivo*. These include BDO₂, 1,2-dihydroxy-3-butene, 1,2-dihydroxy-3,4-epoxybutane, crotonaldehyde, and 1-chloro-3-buten-2-ol. The reactive metabolites, particularly the epoxide compounds and the aldehydes, are likely to be toxic, and it is important to know if they occur *in vivo*, how much is present, and in what tissues. The following are recommended.

- *Determine species and strain differences in the relative rates of formation and subsequent metabolism of potentially toxic metabolites in target and nontarget tissues in humans, monkeys, and rodents using in vitro studies. Also, levels of 1,3-butadiene metabolites following in vivo exposure in the nonhuman species need to be measured.*
- *Determine the metabolism of 1,3-butadiene beyond BDO. How much BDO₂ is formed? How fast is BDO₂ eliminated? Is 1,2-dihydroxy-3,4-epoxybutane an important contributor to toxicity? Is crotonaldehyde formed in vivo?*

There are minimal data on the effect of repeated exposures to 1,3-butadiene or of 1,3-butadiene in mixtures on 1,3-butadiene metabolism, and more data are needed. The induction of various enzymes (notably P-450 2E1) could strongly influence the pattern of the 1,3-butadiene metabolites. Also, there is little information on the variability of 1,3-butadiene metabolism in the human population and how this variability affects the toxicity of 1,3-butadiene for individuals. Therefore, further recommendations are:

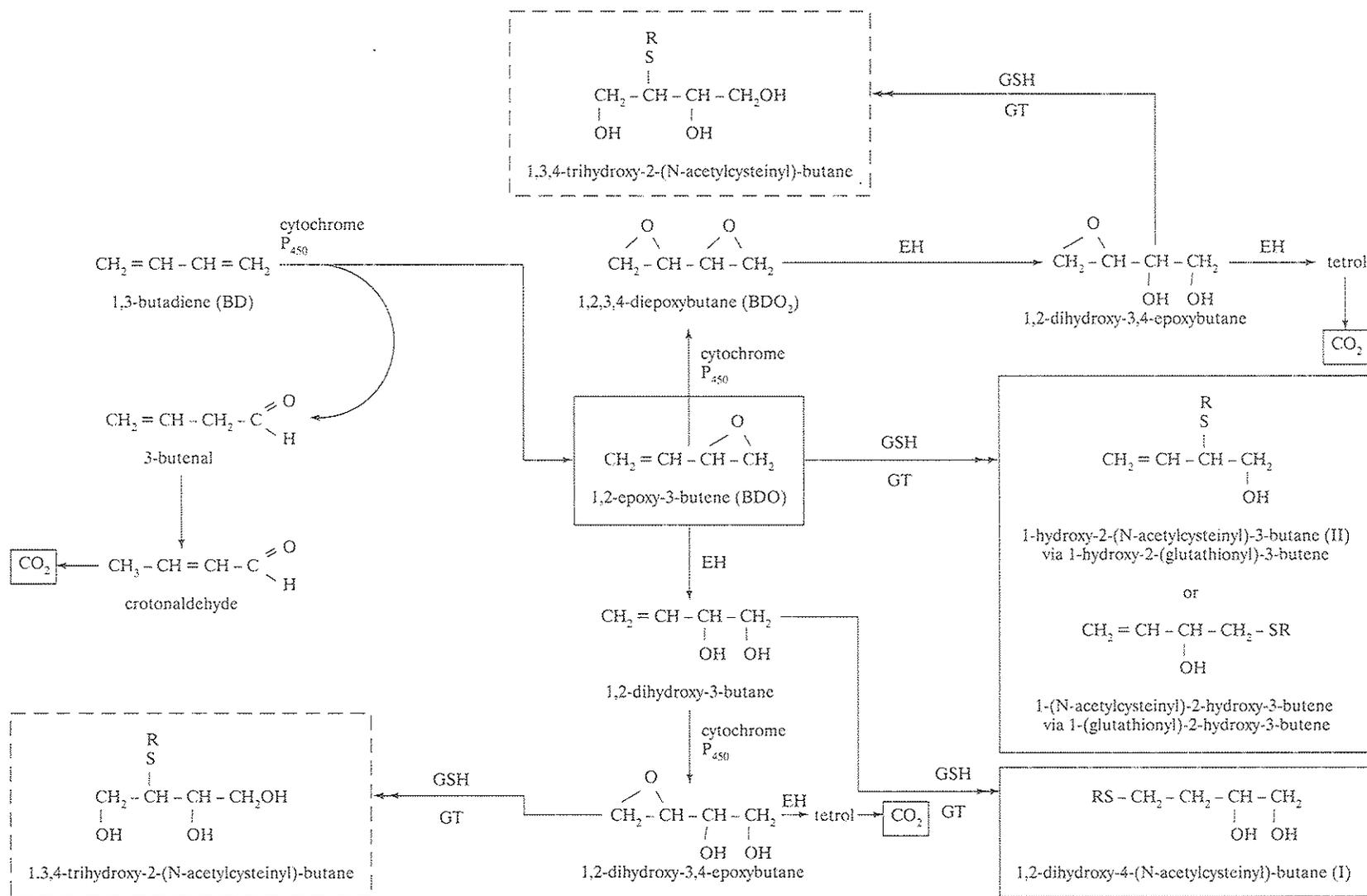


Figure 1. Metabolism of 1,3-Butadiene. Compounds enclosed in boxes have been identified *in vivo* as metabolites of 1,3-butadiene. Tentatively identified compounds are enclosed in broken lines. EH = epoxide hydrolase; GSH = glutathione; GT = glutathione transferase.

- Evaluate the effect of repeated exposures on the metabolism and toxicokinetics of 1,3-butadiene. Do repeated exposures induce the formation of enzymes that will affect the relative rates of formation of toxic metabolites?
- Determine the effect of exposure of 1,3-butadiene in mixtures on the metabolism and toxicokinetics of 1,3-butadiene. For example, does coexposure with styrene competitively inhibit or induce the metabolism of 1,3-butadiene?
- Complete studies to assess tissue doses of 1,3-butadiene, BDO, and BDO₂ in rats, mice, and monkeys exposed to 1,3-butadiene under conditions of linear metabolism and metabolic saturation. Acquisition of these data will be important as physiologically based dosimetry models are developed.
- Develop and validate a physiologically based 1,3-butadiene dosimetry model. The model should allow for predictions of tissue dose of 1,3-butadiene, BDO, and BDO₂ in rats, mice, monkeys, and humans exposed to 1,3-butadiene. These predictions can be tested in nonhuman species in carefully designed laboratory experiments.
- Expand the 1,3-butadiene dosimetry model to include extrahepatic (i.e., site-specific) metabolism of 1,3-butadiene to DNA-reactive epoxides. Data from *in vitro* studies on tissue metabolism of 1,3-butadiene and epoxides will be required.
- Expand the 1,3-butadiene dosimetry model to include other measures of target tissue dose, such as DNA adducts (see below, *Carcinogenicity and Mechanisms of Carcinogenesis*).

ACUTE AND CHRONIC TOXICITY

1,3-Butadiene has long been considered to have a low and noncumulative toxicity in animals and humans. The median lethal concentration (LC₅₀) in rats for a 4-hour exposure was 285 mg/L (i.e., 129,000 ppm or 12.9%) and in mice for a 2-hour exposure was 270 mg/L (i.e., 123,000 ppm or 12.3%) (Shugaev 1969). Early toxicology studies indicated that 1,3-butadiene caused irritation to mucous membranes, skin, and eyes, or narcosis only at high concentrations (Carpenter et al. 1944). Human volunteers exposed to 2,000, 4,000, or 8,000 ppm 1,3-butadiene for 6 to 8 hours

experienced minor irritation to the eyes and difficulty in visual focusing.

Nonneoplastic lesions associated with inhalation exposure of B6C3F₁ mice to 1,3-butadiene for 40 to 65 weeks (Melnick et al. 1990b) included epithelial hyperplasia of the forestomach, endothelial hyperplasia of the heart, alveolar epithelial hyperplasia, hepatocellular necrosis, testicular atrophy, ovarian atrophy, and toxic lesions in nasal tissues (chronic inflammation, fibrosis, osseous and cartilaginous metaplasia, and atrophy of the olfactory epithelium). The proliferative lesions in the forestomach, heart, and lung may represent early preneoplastic changes in the development of 1,3-butadiene-induced neoplasms at these sites. Testicular atrophy was induced at 625 ppm 1,3-butadiene, and ovarian atrophy was observed at concentrations as low as 6.25 ppm.

Concentration-dependent hematologic changes in mice exposed to 1,3-butadiene (62.5–625 ppm) consisted of decreases in red blood cell counts, hemoglobin concentrations, and packed red cell volumes (Irons et al. 1986a, b; Melnick et al. 1990b). These changes were not accompanied by increases in reticulocyte counts or in the frequency of polychromatic erythrocytes in peripheral blood, and may indicate a partial or poorly regenerative response in the bone marrow to reduced levels of circulating erythrocytes. Other changes included an increase in mean corpuscular volume and an increase in mean corpuscular hemoglobin, and mild megaloblastic changes in bone marrow cells. In related studies, Tice and associates (1987) reported that exposure of male B6C3F₁ mice to 1,3-butadiene for 10 days caused a decrease in the number of dividing cells in the bone marrow and a decrease in the rate at which these cells divide, and Thurmond and associates (1986) observed significant extramedullary hematopoiesis in spleens of male B6C3F₁ mice exposed to 1,250 ppm 1,3-butadiene for 24 weeks. One explanation of these results is that exposure to 1,3-butadiene caused suppression of hematopoiesis in the bone marrow, and that young large cells were released into the blood from extramedullary sites, such as the spleen. These findings establish the bone marrow as a target of 1,3-butadiene toxicity in mice.

In a study of hematologic parameters in workers at a styrene-1,3-butadiene synthetic rubber manufacturing plant, slightly lower red blood cell counts, hemoglobin concentrations, and packed red

cell volumes, with higher mean corpuscular red cell volumes, were observed in tank farm workers than in workers in other departments (Checkoway and Williams 1982). The differences in red blood cell indices between tank farm workers and other workers were not statistically significant perhaps because of the small number of workers examined; however, they may be indicative of a 1,3-butadiene-induced effect, because the mean concentration of 1,3-butadiene was about 20 ppm in the tank farm areas and ranged from about 0.1 to 1.0 ppm in the other departments.

No treatment-related gross or microscopic changes or effects on growth, survival, hematologic and blood biochemical parameters, urinary measurements, or neuromuscular functions were observed in male and female Sprague-Dawley rats exposed to 1,3-butadiene at concentrations ranging from 1,000 to 8,000 ppm (6 hours/day, 5 days/week for 13 weeks) (Crouch et al. 1979). Thurmond and associates (1986) reported that exposure of male B6C3F₁ mice to 1,250 ppm 1,3-butadiene (6 hours/day, 5 days/week) for 12 weeks did not produce any persistent defects in humoral or cell-mediated immunity. In a 2-year study, increased mortality in male rats exposed to 8,000 ppm compared with controls was attributed to a more severe nephropathy (Owen et al. 1987).

Exposure of male Swiss CD-1 mice to 200, 1,000, or 5,000 ppm 1,3-butadiene, 6 hours/day for 5 days, followed by cohabitation with untreated female mice for one week, did not affect male fertility, but did cause an increase in the percentage of female mice with 2 or more dead implantations (Morrissey et al. 1990).

In a teratogenicity study, pregnant Sprague-Dawley rats and Swiss CD-1 mice were exposed to 0, 40, 200, or 1,000 ppm of 1,3-butadiene for 6 hours/day, on gestation days 6 to 15. There was no evidence of fetal malformation in either rats or mice. In mice, maternal body weight was reduced in the 200 and 1,000 ppm exposure groups; however, body weights of male fetuses were reduced in the 40, 200, and 1,000 ppm exposure groups. Thus, the male fetus is more susceptible than the dam to inhaled 1,3-butadiene (Morrissey et al. 1990).

CARCINOGENICITY AND MECHANISMS OF CARCINOGENESIS

Carcinogenicity Studies

The carcinogenicity of inhaled 1,3-butadiene was studied in B6C3F₁ mice by the National Toxicology Program (NTP) and in Sprague-Dawley rats by the International Institute of Synthetic Rubber Producers (IISRP). The NTP studies demonstrated that 1,3-butadiene is a potent multiple organ carcinogen. In the initial NTP study, male and female mice were exposed 6 hours/day, 5 days/week, to air containing 0, 625, or 1,250 ppm 1,3-butadiene (Huff et al. 1985, NTP 1984). This study was terminated after 60 to 61 weeks because of reduced survival due to malignant neoplasms at multiple-organ sites. Malignant lymphomas, observed as early as week 20, were the major cause of early deaths in both sexes. In addition, there were early induction and increased incidences of hemangiosarcomas of the heart and neoplasms of the lung and forestomach in males and females, and of the mammary gland, ovary, and liver in females. The high incidence of hemangiosarcomas of the heart was a particularly unusual finding because this neoplasm is extremely uncommon in B6C3F₁ mice.

In an expanded NTP study, male and female mice were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm 1,3-butadiene for up to 2 years (Melnick et al. 1990a). Again, lymphomas were the major cause of early deaths for both sexes exposed to 625 ppm. The incidence of lymphomas was also increased in females exposed to 200 ppm. Hemangiosarcomas of the heart were observed at concentrations as low as 20 ppm in males and 62.5 ppm in females. Incidences of lung neoplasms in female mice were increased at all concentrations, including the 6.25 ppm exposure. The Harderian gland was also identified as a site of 1,3-butadiene-induced neoplasia.

Stop-exposure studies were also conducted in mice to assess the relationship between level and duration of exposures and the outcome of 1,3-butadiene-induced carcinogenicity (Melnick et al. 1990b). These studies demonstrated that tumors were induced at multiple organ sites even after only 13 weeks of exposure to 625 ppm 1,3-butadiene, and that at comparable total exposures, the incidence of lymphoma was greater with exposure to a higher concentration of 1,3-

butadiene for a shorter time (e.g., 625 ppm for 26 weeks) compared with exposure to a lower concentration for a longer time (e.g., 312 ppm for 52 weeks). Thus, for the development of lymphomas, the concentration of 1,3-butadiene is a greater contributing factor than is the duration of exposure.

Irons and associates (1989) reported that a 1-year inhalation exposure to 625 ppm 1,3-butadiene caused a 56% incidence of thymic lymphomas in male B6C3F₁ mice and a 14% incidence in NIH Swiss mice.

In the IISRP study, rats of each sex were exposed to 0, 1,000, or 8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week, for 2 years (Owen et al. 1987). Under these conditions, 1,3-butadiene was carcinogenic at multiple organ sites, as evidenced by increased incidences or dose-response trends for neoplasms of the pancreas and testis in males, and of the mammary gland, thyroid gland, uterus, and Zymbal gland in females. In addition, the average number of mammary gland fibroadenomas per rat was increased in both exposure groups compared with controls. The increased number of uncommon glial cell tumors of the brain in exposed male rats, though not statistically significant, may also have been related to exposure to 1,3-butadiene (Melnick and Huff 1992). These studies reveal that the carcinogenic effects of 1,3-butadiene differ markedly between rats and mice with respect to sites of tumor induction. It is interesting to note that a comparison of tumor sites in animals and in humans shows a similarity between 1,3-butadiene and benzene (Huff et al. 1989).

Mechanisms of Mutagenesis and Carcinogenesis

1,3-Butadiene requires metabolic activation to express a genotoxic response in *in vitro* test systems such as bacteria (deMeester et al. 1980, Poncet et al. 1980) and Chinese hamster ovary (CHO) cells (Sasiadek et al. 1991a). In lymphocytes that have some metabolic capability, 1,3-butadiene produces sister chromatid exchanges (Sasiadek et al. 1991b).

There are differences in species susceptibility to genotoxic effects of 1,3-butadiene; in the mouse, sister chromatid exchange and chromosomal aberrations were induced in bone marrow (Cunningham et al. 1986, Irons et al. 1987a, Tice

et al. 1987). The incidence of micronuclei occurring in peripheral lymphocytes and bone marrow was also increased in 1,3-butadiene-exposed mice (Cunningham et al. 1986, Tice et al. 1987, Jauhar et al. 1988), but these effects were not seen in rat bone marrow cells (Cunningham et al. 1986).

Protein-DNA and DNA-DNA cross-links have been found in livers of 1,3-butadiene-exposed mice. No such cross-links were detected in the rat (Jelitto et al. 1989).

Metabolism studies suggest it is likely that one ultimate carcinogen of 1,3-butadiene is BDO. This reaction appears to be catalyzed predominantly by cytochrome P-450 2E1 (Csanády et al. 1992, International Agency for Research on Cancer 1992). BDO is genotoxic and tumorigenic (International Agency for Research on Cancer 1992). It reacts with DNA to produce two identified adducts, 7-(2-hydroxy-3-buten-1-yl)guanine and 7-(1-hydroxy-3-buten-2-yl)guanine (Citi et al. 1984). The presence of the former along with 7-*N*-(2,3,4-trihydroxybutyl)guanine has been reported in the livers of mice exposed to ¹⁴C-1,3-butadiene (Jelitto et al. 1989).

Further oxidation of BDO produces BDO₂. This reaction has been reported but occurs less readily than the formation of BDO from 1,3-butadiene (Csanády et al. 1992, International Agency for Research on Cancer 1992). BDO₂ is genotoxic and carcinogenic and could thus potentially play a role in 1,3-butadiene carcinogenicity (International Agency for Research on Cancer 1992). BDO₂ DNA adducts have not been characterized. While 1,3-butadiene has effects in human lymphocytes *in vitro*, its epoxide metabolites are genotoxic to lymphocytes at concentrations approximately 50 times lower (BDO) or 1,000 times lower (BDO₂) than 1,3-butadiene concentrations (Sasiadek et al. 1991b).

Crotonaldehyde has also been reported as a metabolite of 1,3-butadiene (Elfarra et al. 1991). Crotonaldehyde is mutagenic and carcinogenic and forms cyclic 1,*N*-²propanodeoxyguanosine adducts *in vitro* and *in vivo* upon reaction with DNA (Chung et al. 1984, 1986, 1989).

Recio and associates (1992) recently conducted a study to measure the frequency of 1,3-butadiene mutations in mouse tissues using a transgenic mouse. This transgenic mouse (BALB/c × DBA/2) has a bacteriophage λ shuttle vector with

the target gene *lac z* integrated into the mouse genome. Mice were exposed by inhalation to 625 ppm 1,3-butadiene (6 hours/day) for 5 days, and the *lac z* mutant frequency was determined in lung, bone marrow, and liver. The *lac z* mutant frequency was increased two-fold in lung samples compared with air-exposed controls; however, the bone marrow and liver samples did not exhibit an increase over controls. These results indicate that 1,3-butadiene induces mutations *in vivo* in a known murine target tissue.

At least two aspects of 1,3-butadiene carcinogenesis in the mouse are intriguing. First, the incidence of 1,3-butadiene-induced murine thymic lymphoma or leukemia is remarkably high in a mouse strain that possesses ecotropic murine leukemia virus (Irons et al. 1987b, 1989; Irons 1990). These tumors are of thymic origin, express T cell antigens, and possess T cell receptor gene arrangements indicative of differentiated T cells. Curiously, these tumors exhibit an autocrine cytokine dependence (interleukin-2), reminiscent of HTLV-I-induced adult T cell leukemia in humans. Second, although chronic exposure to 62.5 ppm or higher concentrations of 1,3-butadiene is associated with a macrocytic anemia in the mouse, the overall acute and subacute toxicity of 1,3-butadiene is remarkably low in all species including humans.

In a pilot study reported in abstract form (Ward et al. 1992), the effect of 1,3-butadiene exposure on the frequency of T cell lymphocytes containing mutations at the hypoxanthine guanine phosphoribosyl transferase (*hprt*) locus was assessed. In 7 workers exposed to 1,3-butadiene (3.5 ± 7.5 ppm), there was a statistically significant increase in the variant frequency of lymphocytes compared with the low-exposure group (0.03 ± 0.03 ppm) and with control workers. These workers were nonsmokers, but the elevation in the frequency of *hprt* mutations in the high-exposure group was similar to that observed in cigarette smokers. No information was given concerning the previous work exposure histories of the workers in the "high"-exposure group. Cytogenetic analyses, including chromosomal aberrations, sister chromatid exchange, and micronucleus induction, have been conducted on workers exposed to air concentrations of up to 10 ppm 1,3-butadiene. These have shown no differences between these workers and an unexposed referent worker group (Sorsa 1992).

The role of *ras* oncogene mutations in development of either human or murine leukemia requires further study. The proportion of *ras*-positive leukemias or lymphomas in individual studies of murine models rarely exceeds 60%. Activated *K-ras* genes have been detected in less than 20% of murine lymphomas associated with 1,3-butadiene exposure (Goodrow et al. 1990).

Activated *K-ras* oncogenes were also detected in 6 of 9 lung adenomas and 3 of 12 hepatocellular carcinomas induced by 1,3-butadiene in B6C3F₁ mice. A G-to-C transversion in codon 13 was a common mutation (Goodrow et al. 1990). The presence of p53 mutations in mouse lung and mammary gland tumors induced by 1,3-butadiene has also been noted (Soderkvist et al. 1992).

Research Recommendations. On the basis of the literature and experience with other related carcinogens, the simplest assumption would be that DNA modification by electrophilic metabolites of 1,3-butadiene plays a significant role in its mechanism of carcinogenesis. 1,3-Butadiene monoxide would be a probable ultimate carcinogen. Yet the interactions of this compound with DNA have been examined only superficially to date. In addition, there is no information on the repair or biological significance of the adducts that are formed. Few data are available on DNA modifications *in vivo* by 1,3-butadiene itself. There are also limited data on DNA modifications by other potential ultimate carcinogens of 1,3-butadiene including BDO₂ and crotonaldehyde. Therefore, the following are recommended:

- Identify the DNA adducts produced *in vitro* by all potential ultimate carcinogens of 1,3-butadiene including BDO, BDO₂, and crotonaldehyde. Identify the adducts produced *in vivo* by 1,3-butadiene in the target and nontarget tissues of mice and rats.
- Assess the *in vivo* persistence and repair of these adducts in target and nontarget tissues of 1,3-butadiene-treated animals.

In addition, no methods are currently available to assess 1,3-butadiene-DNA modifications in humans.

- Develop sensitive methods to detect these DNA adducts in human tissues or body fluids. Carry out analogous studies with surrogate macromolecules such as hemoglobin.

The above information would aid in understanding the origin and significance of mutations in the *K-ras* and *p53* genes as analyzed in 1,3-butadiene-induced tumors. Analysis of such mutations in human tissues could help in risk assessment.

- *Compare the patterns of mutations in activated oncogenes or inactivated suppressor genes of tumors induced in animals by 1,3-butadiene with those in tumors from humans with suspected exposures to 1,3-butadiene.*

The factors that distinguish site-specificity and exposure-response differences for 1,3-butadiene-induced carcinogenesis among species need to be identified. We also need to determine how 1,3-butadiene-induced cancer is affected by host-determined intrinsic factors (smoking, alcohol use, diet, exposure to other chemicals including medication and recreational drugs, etc.).

The possible relationship between the elevated incidence of leukemia/lymphoma in the mouse and the apparently increased incidence of lymphosarcoma or reticulosarcoma in one 1,3-butadiene manufacturing plant (Divine 1990) is intriguing and deserves further study. However, the etiology of murine thymic lymphoma/leukemia is complex, and the possible role of murine leukemia virus in chemically and radiation-induced murine leukemia has been recognized for almost half a century (Upton et al. 1958, Jandl 1987).

- *Because the carcinogenic endpoints variously implicated in 1,3-butadiene exposure in humans involve the lymphocytic or hematopoietic systems, elucidation of the mechanisms of chemical leukemogenesis both in humans and in the mouse is important with respect to determining the relevance of this animal tumor model for extrapolation to humans.*

EPIDEMIOLOGY

The existing epidemiologic studies that can be used to evaluate the effects of exposure to 1,3-butadiene involve two separate industries: one study is from the 1,3-butadiene manufacturing industry, and three are from the rubber industry. The characterization of exposure in all of the plants in which 1,3-butadiene was involved was inadequate, particularly before the 1980's. However, it is

generally recognized that exposures were higher in the earlier years of 1,3-butadiene usage.

One cohort of workers from a 1,3-butadiene manufacturing plant consisted of 2,582 men who had worked for 6 months or more in a single plant at some time during the period 1943 to 1979. The original study of the workers (Downs et al. 1987) reported an excess of lymphosarcoma and reticulum cell sarcoma in the population which had 8 deaths versus 3.4 expected on the basis of U.S. rates or 4.4 expected on the basis of local rates. Divine (1990) updated the mortality of this cohort through 1985 and the lymphosarcoma mortality was 9 observed, 3.9 expected (standardized mortality ratio, SMR = 2.29) on the basis of U.S. rates. The lymphosarcoma excess did not increase with duration of employment. However, the risk appeared to be higher for those employed before 1946 (SMR = 2.69 vs. SMR = 1.55 after World War II) and for those who had "routine" exposure to 1,3-butadiene (SMR = 5.61), though for routinely exposed workers employed more than 10 years no lymphosarcoma deaths were recorded (0.4 expected). The workers in this group had jobs in processing, laboratory work, receiving, storage, and transport.

Leukemia showed no significant excess in the total population (8 cases observed, 7.9 expected) or in those with routine exposure (1 observed, 1.8 expected). Five of the 8 leukemia cases occurred in workers who had been employed in jobs with nonroutine exposures and who had less than 10 years of employment (vs. 1.7 expected). The SMR of 2.94 in this group approached significance ($p = 0.06$). Workers with non-routine exposures of 10 years or more had one leukemia observed versus 1.5 expected. Although no SMRs for other cancer sites reached significance, the authors have noted that several cancers appeared to occur with SMRs above 1.00 for workers who were first employed after 1946. The cancers included lymphosarcoma, Hodgkin's disease, and cancers of other lymphatic tissue, large intestine, pancreas, and kidney. The SMR for pneumonia was 1.48. The investigators suggested that the pre-World War II period may have high risks because of differences in hiring practices and transfer of individuals from other industries into the new facilities. Although the population is small, this cohort is the largest reported involved in the manufacture of 1,3-butadiene.

The cohort of Meinhardt and associates (1982) consisted of 2,756 white males who worked 6 months or more in two plants that manufactured styrene-1,3-butadiene polymer. Plant A had records from the beginning of operations in 1943, but records were incomplete for plant B. All workers were followed through 1976. Workers in plant A had an excess of leukemia and lymphatic and hematopoietic cancers, which was confined to those who had started work between 1943 and 1945. The SMR for leukemia for this group of workers was 2.78 (5 observed, 1.8 expected), and this was significant using a one-sided test. The researchers reported no excesses in death rates for plant B, in which the study cohort did not start until 1950. Leukemia findings for plant B were 1 case observed, 1 expected. Leukemia SMRs did not show discernible patterns associated with duration of employment or time since first employment. However, for the styrene-1,3-butadiene rubber (SBR) industry, duration of employment is only a crude surrogate for exposure.

The 12,110 male workers in the cohort of Matanoski and associates (1990) represent employees from eight facilities in the United States and Canada that manufacture SBR polymer as well as other synthetic rubbers. The cohort consists of all workers who were employed for one year or more from the start of complete record keeping in each plant. The population has been followed to 1982. The total cohort shows no excess health risk except for significantly elevated SMRs for circulatory disease deaths (SMR = 1.18; 95% confidence interval [CI] 1.03–1.35) and arteriosclerotic heart disease deaths (SMR = 1.48, 95% CI 1.23–1.76) in black workers. The SMRs were elevated for several cancers, especially for black males, but the excesses were not significant. When the population was divided into work areas, the SMRs in the production area for leukemia and for lymphatic and hematopoietic cancers in black workers were significantly elevated (3 observed, SMR = 6.56, 95% CI 1.35–19.06, and 6 observed, SMR = 5.07, 95% CI 1.87–11.07, respectively). White production workers showed 4 observed, 4.8 expected (SMR = 0.84) for leukemia, and 13 observed, 11.8 expected (SMR = 1.10) for all lymphopoietic cancer. The SMR for other lymphatic cancers in all production workers was 2.60 (95% CI 1.19–4.94). Nonsignificant excesses of these same cancer groups occurred in utility

workers and "other area workers," but not among mechanical workers. White male production workers had an elevated SMR for kidney cancer (SMR = 1.66, 95% CI 0.54–3.88). Maintenance workers showed a nonsignificant elevation of risk for stomach cancer (18 observed, 12 expected, SMR = 1.51), but not for other digestive cancers. The "other area worker" category had an excess risk of rheumatic heart disease among the white males (SMR = 2.09, 95% CI 1.08–3.65). The production and maintenance workers were thought to be most exposed to 1,3-butadiene (Fajen 1990), although selected workers in all work areas might also have exposure.

As a follow-up to this study, all cases of lymphatic and hematopoietic cancers identified from all conditions listed on the death certificate were included in a nested case-control study. All jobs were ranked for both styrene and 1,3-butadiene exposure by a group of engineers from the industry. The cases were matched to controls by date of birth, date of hire, plant, and duration worked, with a final control:case ratio of 3.3:1.0. The cumulative exposure score for each worker was based on the estimated rank for each job and the number of months in that job summed over all jobs held during the total period of employment. Using the mean of the log rank scores of the controls to define exposure, the estimated risk of leukemia associated with 1,3-butadiene exposure corrected for styrene exposure was 7.4 (95% CI 1.32–41.3) (based on the odds ratio from a conditional logistic regression model). There were 26 leukemia cases in the analysis. When a variable was added to include work in operation services, laboratory, and utilities, these jobs were also associated with an increased risk of leukemia independent of the risk from the estimated 1,3-butadiene exposure. There was little risk of other specific cancers in this category associated with exposure to either chemical. The overall category showed an increased risk associated with 1,3-butadiene exposure (SMR, 2.42 95% CI 1.12–5.23), probably reflecting the elevated risk associated with leukemia in this group. The leukemia risk appeared to increase with increasing log exposure score, suggesting a "dose" response even with the use of this crude exposure measure (Santos-Burgoa et al. 1992).

The analysis and interpretation of this study are points of controversy. Cole and associates (1993) presented a reanalysis of this study that showed

much lower odds ratios based on only slightly different cutpoints and the lack of an exposure-response relationship with fewer exposure categories (3 and 4 categories) than were used in the report by Santos-Burgoa and associates (7 categories). Cole and associates (1993) and Acquavella (1989) have also questioned the high odds ratio of 7.4 in light of the findings for the total cohort of 22 observed leukemias versus 23 expected.

McMichael and associates (1976) found excesses of deaths by several causes including cancers of the stomach, colon, prostate, lymphosarcoma, Hodgkin's disease, and leukemia in a cohort of 6,678 male workers in a tire-manufacturing plant for the period 1964 to 1973. They conducted a nested or hybrid case-control study to determine the possible association of any of these cancers with work in any specific area. The investigators included only jobs that the workers had held at least two years or more in the period 1940 to 1960, which represented an average latency of 8 to 29 years to the onset of cancer. The results of the study indicated that there was a significant association between work in the synthetic rubber latex plant and excess risk of stomach cancer (risk ratio [RR] = 2.2, 99.9% CI 1.4-4.3), of lymphatic and hematopoietic cancer (RR = 6.2, 99.9% CI 4.1-12.5), of lymphatic leukemia (RR = 3.9, 99.9% CI 2.6-8.0) and of ischemic heart disease (RR = 3.0, 99.9% CI 1.7-12.5). The authors indicated that the leukemia risk was also associated with solvent exposure in work areas other than the synthetic plant. They noted that the high risk in the overall category of lymphatic and hematopoietic cancers must indicate an even higher association of cancers other than leukemia in the group with exposure in the synthetic latex plant, but they do not provide further analysis of the subgroups. Clarification of this finding with respect to 1,3-butadiene has not been forthcoming, but Smith (1977) reported that the same association was not found in a similar synthetic plant under study or in an ongoing investigation in a third synthetic plant, but this may be due to number of subjects studied.

A population-based case-control study of multiple cancers except leukemia has indicated an increased odds ratio for kidney cancer in association with exposure to styrene-1,3-butadiene rubber (odds ratio 2.0, 90% CI 1.2-3.4) based on 12 cases. There was no significant association of

non-Hodgkin's lymphoma although the odds ratio was 1.5 (90% CI 0.4-5.1) for exposures classified as "substantial" (Siemiatycki 1991).

Research Recommendations.

Exposure. Because retrospective exposure estimation has inherent uncertainties, there is a need to improve estimates of past exposures in existing study populations. For example, personnel job records do not indicate differences in exposure for workers with the same job title but different tasks. This problem is currently being addressed. The International Institute of Synthetic Rubber Producers has sponsored a retrospective cohort study with quantitative exposure estimation for each cohort member over the entire study period from 1942 to 1991 for the study by Matanoski and associates (1990) and for the two SBR plants studied by Meinhardt and associates (1982). The exposure estimation methodology will consist of process analysis, job analysis, exposure estimation, and linkage with specific jobs in workers' histories. Completion of this study is expected in late 1994.

Continued evaluations of existing populations are useful, but foreign populations should be considered because of the high doses that probably exist in lesser developed countries such as China. Such populations would offer the opportunity to measure exposure and some aspects of worker response concurrently as well as to look retrospectively at a population with particularly high exposures. Emphasis might be placed on the evaluation of lymphopoietic cancer rates, hematologic effects, biomarker research, arteriosclerosis, and evaluation of reproductive endpoints. The role of coexposure to other chemicals is also unclear. The following are important research recommendations.

- *Add new populations at higher exposures to expand and confirm the findings in the limited number of populations currently under study and to allow evaluation of biomarkers, pathology, gene mutations, and reproductive outcomes.*
- *Determine the exposure of study populations using both estimated and measured data. Explore the glycophorin polymorphism in red blood cells as a long-term biomarker of exposure.*
- *Coexposure to chemicals that may affect the metabolism of 1,3-butadiene should be examined as part of an effort to explain the differences in*

cancer response in the different industries using 1,3-butadiene. Coexposure to other carcinogens also should be examined.

Improving Diagnostic Information. The studies to date have focused on the underlying cause of death on death certificates. Lymphopietic cancers may be misdiagnosed because the classification is complex. For example, lymphosarcoma is now included under non-Hodgkin's lymphoma as a diagnosis in cancer registries. Lymphopietic cancers may also occur in conjunction with immunosuppressive diseases and with treatment for other diseases. The populations currently under study need confirmation of the diagnoses of cancer as well as other diseases from hospital records wherever possible to strengthen the pathologic and biologic information regarding the cases. Further pathologic confirmation of cases by a panel of pathologists would be desirable since the studies involve geographic and time differences that could result in inconsistent diagnoses. Therefore, an important research recommendation is:

- *Confirm cancer diagnoses in ongoing and future epidemiology studies.*

It is important to extend the epidemiologic studies, where feasible, to include new analysis methods for studies of tumor tissue. For example, specific mutations have been seen in the p53 gene in association with skin cancer. Animal studies have suggested that specific abnormalities in codons of the *ras* oncogene may be unique to certain chemicals. Pursuit of such abnormalities may be useful in human studies because it might give us a more specific endpoint for risk assessment.

The emphasis to date has been on the epidemiology of cancers of the lymphatic and hematopoietic system because of the findings in early studies in mice at high exposures. However, later studies indicated that at lower doses other cancers became evident.

RISK ASSESSMENT

The EPA classified 1,3-butadiene as a "probable human carcinogen" (group 2B) on the basis of two rodent inhalation studies. Using a linearized multistage dose extrapolation model and other usual assumptions, 1,3-butadiene's upper bound carcinogenic potency has been estimated by the

EPA to be 0.25 ppm^{-1} . The International Agency for Research on Cancer (1992) has given 1,3-butadiene an overall evaluation of 2A: "probably carcinogenic to humans." It concluded that there is limited evidence of human carcinogenicity of 1,3-butadiene on the basis of the most recent studies of Downs and associates (1987), Divine (1990), and Matanoski and associates (1990).

Since the EPA's 1985 Health Assessment, a number of studies have been published that bear directly on cancer risk assessment. It appears that the mechanism of the oncogenic activity shown by 1,3-butadiene in rodent bioassays and possibly in humans is related to its genetic toxicity. The most detailed evaluation of the carcinogenicity of 1,3-butadiene is the mouse inhalation study sponsored by the National Toxicology Program (Melnick et al. 1990b). This study did not establish a no effect level for reproductive endpoints. The 6.25 ppm nominal dose level might be considered a chronic lowest observable adverse effect level (LOAEL) for reproductive toxicity. With respect to quantitative risk assessment, the epidemiologic data base is still considered inadequate for predicting risks of community exposure to 1,3-butadiene; hence risk assessment must rely on rodent cancer bioassay data. In particular, the study reported in Melnick and associates (1990b) was considered most appropriate by California EPA because of the high sensitivity of the tumor responses.

In the California EPA risk assessment, the potency of carcinogenic response in animal studies was determined by converting the nominal doses to (1) measured applied doses; (2) continuous internal doses using the absorption data of Bond and associates (1986); and (3) the metabolized doses from the pharmacokinetic analysis of Hattis and Wasson (1987). Also, a modified physiologically based pharmacokinetic (PBPK) model similar to that of Hattis and Wasson was used to estimate a blood epoxide dose.

Cancer potency estimates by California EPA were made for mice and rats using both total significant tumor incidences and tumor incidences at individual sites, several approaches to measuring dose, and the linearized multistage model of low-dose extrapolation. The most sensitive tumor site was the lung, where alveolar and bronchiolar neoplasms were observed in female mice. The continuous internal dose was considered to be the best measure of dose available. When interspecies

equivalent units of milligrams per unit surface area were used, an example of the upper range of human cancer potency based on rat and mouse assays was 9.8×10^{-3} to 0.8 ppm^{-1} . That is, for each ppm of a 24 hour, 70-year lifetime exposure, risk ranges from 1% to 80%.

Using the upper-bound extra cancer risk based on the evidence of lung neoplasms in females in the mouse study, a mouse-based human cancer potency of 0.37 ppm^{-1} or $3.4 \text{ (mg/kg-d)}^{-1}$, with a unit risk value of $1.7 \times 10^{-4} \text{ (}\mu\text{g/m}^3\text{)}^{-1}$ can be derived. Exposure to 6 ng/m^3 is associated with a lifetime excess cancer risk of 1×10^{-6} . Based on an average California ambient concentration of 0.37 ppb and a cancer potency of 0.37 (ppm)^{-1} the resulting lifetime upper bound population risk is $1.4 \times 10^{-4} (0.37 \text{ ppb} \times 0.37 \times 10^{-3} \text{ ppb}^{-1})$.

The choice of animal model to predict effects in humans is an issue of concern. Species differences in the rates of metabolism of 1,3-butadiene and its metabolites (see above, Biochemistry, Metabolism, and Toxicokinetics) suggest that the mouse may be more efficient at toxication reactions in the metabolism of 1,3-butadiene and less efficient at detoxication reactions than are other species studied. The B6C3F₁ mouse also has an endogenous retrovirus that may enhance the increase in malignant lymphomas induced by 1,3-butadiene exposure in that strain (Irons et al. 1987b, 1989). Nevertheless, the findings of lymphatic system neoplasms in the studies of Downs and associates (1987) and of Matanoski and Schwartz (1987) appear analogous to the tumor type induced in the mouse at high exposure concentrations.

Research Recommendations.

There are a number of uncertainties in the risk assessment of 1,3-butadiene. Most of the issues are typical of those found in risk assessment, but a few are specific to 1,3-butadiene. One issue is the apparently large range of risks associated with 1,3-butadiene depending on the species or tissue used for risk assessment. A related issue is whether this range of risk would hold if target tissue dose were available. The multiplicity of tumor sites is another issue indicating that possibly a combined tumor approach should be used in 1,3-butadiene risk assessment.

With regard to dose-response extrapolation, the competing risks among tumor sites need to be taken into account. Currently, the range of

extrapolation of tested dose to environmental levels is 1,000-fold.

- *A comprehensive time-to-tumor evaluation could possibly help resolve the issue.*

The health impact of 1,3-butadiene to workers exposed to current occupational levels needs to be better defined.

- *It should be determined whether cytogenetic damage or mutations are detectable as biomarkers of exposure, for example, in lymphocytes of humans exposed.*

Information on 1,3-butadiene with regard to tissue dose modeling is insufficient. The distribution of 1,3-butadiene and key metabolites in humans is not known. The metabolism of 1,3-butadiene and production of key metabolites in blood, kidney, liver, breast, lung, and other tissues is also not known.

- *Dose-response models should be developed for short-term exposure, considering peak tissue epoxide levels and total area under the dose curve. Quantitative risk assessment procedures should be developed for analysis of short-term exposures that consider the relationship between dose and duration.*

There is an apparent lack of correspondence between measured 1,3-butadiene metabolite concentration in tissues and the subsequent tumor incidence in these tissues. This inconsistency needs to be evaluated to determine if it is due to tissue sensitivity, repair capacity, detoxification, or lack of understanding of mechanism of action.

- *The physiologically based pharmacokinetic models should be evaluated to determine if tissue dose can explain tumor production in various tissues.*

The impact of 1,3-butadiene on humans with regard to reproductive effects is unclear, particularly the production of abnormal sperm morphology, gonadal atrophy, and fetotoxicity in mice.

PRIORITIZATION OF RESEARCH RECOMMENDATIONS

Metabolism and Toxicokinetics

We know the broad outlines of the metabolic processing of 1,3-butadiene, but we do not know enough to be sure we have a grasp of all the

potentially toxic metabolites and their relative importance. Therefore, we need a more detailed understanding of animal and human metabolism and the nature and magnitude of variability in metabolism.

Various organs differ in their rates and patterns of 1,3-butadiene metabolism. The toxic alkylating metabolites of 1,3-butadiene, for example BDO, are relatively long-lived, so the dose to any given site is a function of the local metabolite formation and transport of metabolites to that site. Therefore, we need pharmacokinetic models of 1,3-butadiene to predict local tissue dosage of 1,3-butadiene and its metabolic intermediates for various exposure scenarios.

Genotoxic and Carcinogenic Mechanisms

We have only a sketchy idea of the pharmacokinetics of 1,3-butadiene-induced DNA adduct formation, that is, the rates and sites of adduct formation and removal in different tissues and their consequent genotoxic effects. Therefore, we need a model that would permit the quantitative characterization of somatic cancer-related genotoxic injury as well as genetic damage per se for relevant exposure scenarios.

Given the epidemiologic evidence that lymphatic or hematopoietic cancers are possible responses of humans to 1,3-butadiene, studies using the mouse lymphoma model have been used in risk assessment. However, we are unsure on mechanistic grounds that this model is relevant to humans. Therefore, we need information that would indicate whether key steps in the carcinogenic process, particularly in lymphatic or hematopoietic tissues are the same in mice and humans. However, it should be recognized that other tumor types induced in rodents may be important in human tumorigenesis by 1,3-butadiene, so a broader understanding of tumorigenic mechanisms would be desirable.

Epidemiology

Epidemiology studies have shown an increase in lymphopoietic cancers in subgroups of workers exposed to 1,3-butadiene in occupational settings. Lymphopoietic cancer has occurred predominantly in workers with a short period of exposure and not in workers with many years of exposure. This unusual epidemiologic response pattern requires

explanation. The response might reflect, for example, some unidentified confounding factors, a sensitive population that leaves the industry in a short time, or high exposure levels only in the early periods of employment. Therefore, we need to study other highly exposed industrial populations in which reliable and detailed exposure patterns can be obtained with respect to the type and timing of cancer induction. Such populations might be sought in Eastern Europe or China. These populations could also provide information on fertility outcomes and developmental effects.

Research Priorities

In view of these facts, the 1,3-butadiene working group recommends the following as the major research needs having the highest priorities for advancing the scientific basis of 1,3-butadiene risk assessment (not in order of priority). The full text discusses all research needs as determined by the working group. However, the six recommendations below have the highest priority, and significant results may be expected in 3 to 5 years.

- *Determine in vitro and in vivo species differences in the relative rates of formation and subsequent metabolism of potentially toxic metabolites (most likely, the epoxides and perhaps crotonaldehyde) in target tissues (liver, heart, lung, bone marrow, lymphatic tissue) and nontarget tissue in humans, nonhuman primates, and rodents.*
- *Complete the development and validation of a physiologically based 1,3-butadiene dosimetry model. The model should allow for predictions of target and nontarget tissue dose of 1,3-butadiene and epoxides in rats, mice, nonhuman primates, and humans. These predictions can be tested in carefully designed laboratory experiments. Carry out these studies to assess target tissue dose of 1,3-butadiene and epoxides in rats, mice, and nonhuman primates exposed to 1,3-butadiene under conditions of linear metabolism and metabolic saturation.*
- *Determine whether 1,3-butadiene induces mutations and, if so, the frequency and mutational spectra in relevant tissues of laboratory animals and humans.*
- *Identify the cancer-related DNA adducts produced by potential ultimate carcinogens of 1,3-butadiene and by 1,3-butadiene itself. Develop sensitive methods to detect these adducts in human body fluids or tissues. Carry*

- out analogous studies with surrogate macromolecules such as hemoglobin and urinary metabolites.* These studies will provide biomarkers for assessing uptake and metabolism of 1,3-butadiene as well as providing mechanistic insights on 1,3-butadiene carcinogenesis, particularly in the case of metabolism. These studies will also yield information on the variability of 1,3-butadiene metabolism in humans.
- *Because the carcinogenic endpoints variously implicated in 1,3-butadiene exposure in humans involve the lymphocytic or hematopoietic systems, elucidation of the mechanisms of chemical leukemogenesis in both humans and mice is important with respect to determining the relevance of this animal tumor model for extrapolation to humans. To improve the accuracy of the risk assessment process, a model of tumor development should be an ultimate goal. An important aspect of this approach is an understanding of the species differences in tumor types observed and their mechanisms of causation.*
 - *Carry out epidemiology studies on new populations at higher exposures to expand and confirm the findings in the limited number of populations currently under study and to allow evaluation of biomarkers and reproductive outcomes.*
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Research Priorities to Reduce Uncertainties in Risk Assessment for Methanol

INTRODUCTION

The projected widespread use of methanol fuels poses an array of questions about the potential for health risks. Some of these questions stem from the variety of possible exposure scenarios. The entire chain of potential human contact preceding vehicle emissions, from production and transport to tank filling, needs to be considered in determining health risks.

Although the predominant route of exposure is likely to be inhalation of methanol vapor, dermal exposure and even ingestion must be considered as well. Environmental exposures are predicted to be limited to low concentrations. However, accidental exposures arising from the sequence of processing, from production to combustion, carry the risk of significantly higher exposure levels. Deliberate intakes can also not be ruled out. Given this potential for exposure in different scenarios by a variety of routes, the exposure-dose-response question should be expanded to include a wide range of exposures. As exposure levels rise, the pattern of biological effects shifts and engenders a different series of toxicologic questions, as reviewed in this report.

Research Recommendations. Because of the need to consider the range of toxic outcomes extending from low to high exposure levels, research priorities do not neatly coincide with the size of the populations corresponding to these levels.

For example, only high levels of methanol produce marked elevations of formate, the assumed proximate toxicant in methanol poisoning. If our experience with gasoline is any guide, adventitious exposures to high levels are inevitable. Hospital emergency room records regularly identify cases of deliberate ingestion by

young children or accidental ingestion during siphoning. Another concern is the possibility that many more individuals may be exposed repeatedly to moderate levels, with consequences that are more subtle than those of outright methanol poisoning. Transitory elevations in formate levels might ensue from such exposures. Even exposure levels currently acceptable in the workplace remain to be conclusively demonstrated as free of adverse effects. Finally, the enhanced susceptibility of the developing organism to many other chemical agents, including ethanol, mandates a thorough examination of methanol effects during this stage of life. The research recommendations briefly summarized below, and explained in more detail in the body of the report, have been guided by the need to account for a wide range of levels of exposure.

- *Models designed to estimate airborne exposure concentrations along the distribution pathways for methanol need to be developed, along with ambient and personal monitoring data to validate such models. Dermal routes should also be examined.*
- *More data relating ambient methanol concentrations to methanol and formate blood levels are required to formulate pharmacokinetic models. Such models are useful in two contexts. First, to predict tissue levels of methanol as a function of exposure concentration in both humans and in laboratory animals. Second, to devise models relating methanol exposure to formate accumulation.*
- *Major efforts are called for in defining health risks. Their objective should be to link exposure, dose, mechanisms, and effects in a quantitative framework. Experimental human exposures would help provide a comprehensive assessment of adverse neurobehavioral consequences. Estimated risks to the fetus are based on only a handful of studies in rodents; such studies should be extended, should include nonhuman primates, and should also deal with the contribution of folate status.*
- *The mechanism and measurement of ocular toxicity, the most pronounced aftermath of human poisoning, require further investigation at lower doses. Promising animal models have been devised that deserve further exploitation.*

Methanol Working Group: Bernard Weiss (Chair), Gareth M. Green (Rapporteur), Thomas M. Burbacher, Maria G. Costantini, Janis T. Eeels, Eun Woo Lee, Paul J. Liroy, Michele A. Medinsky, and John M. Rogers.

- *The question of chronic exposure needs to be addressed both experimentally and, if possible, epidemiologically.*

EXPOSURE ASSESSMENT

The adoption of methanol as an alternative fuel presents a challenge to the fields of exposure analysis and risk assessment. Because methanol is not yet widely distributed, there is the opportunity to develop a model approach that can prescribe, before widespread use, exposure standards for methanol fuel or its combustion products based on scientific information. The basic framework includes analysis of the situations in which contact can occur, both for members of the general population and for individuals in occupational settings, and studies to monitor exposures in situations in which methanol is currently used. Concurrently, possible biological effects must be identified to ensure that the dimensions of exposure can be used to predict the potential for acute, subacute, or chronic effects (Lioy 1992). These subjects typically are dealt with separately, a practice that hampers the identification of potentially adverse exposures and, ultimately, risk.

Another problem relevant to risk assessment is that methanol will probably be manufactured and offered to the public in combination with other materials (methanol-gasoline and ethanol-methanol-gasoline blends are examples). Potential exposure scenarios should consider methanol combinations with other materials, primarily gasoline (Wixton and Brown 1992), because it seems most likely that this combination will predominate. Further, the mixture of evaporative and combustion pollutants from these fuels must be considered when defining exposures for the general population.

The Distribution Pathway: Routes of Exposure and Exposure Scenarios

Table 1 describes the major settings that may lead to exposure from methanol fuel. At critical stages in the analysis of emission sources, we must determine if each point of potential contact, which includes inhalation, dermal, and ingestion routes of exposures, typically represents low, moderate, or high exposures and couple these relationships to expected effects. Such an analysis provides the

means for modeling exposures from fuel transfer through end uses to disposal. It can also be used to identify the control strategies required to minimize the possibility for both direct and indirect exposures. Although incidental and accidental exposures are certain to occur, the controls needed for fuel dispensing, vapor retention, and emissions reduction should be flexible enough to ensure minimal exposures under normal operating conditions. Examples of the types of source-receptor relationships for identifying the routes of exposure to methanol can be derived from gasoline fuel use (Guldbers 1992, Weisel et al 1992).

Exposure of the general public to methanol fuel will be dominated by the inhalation and dermal routes. For the inhalation route (Table 1) the microenvironments of concern include the general ambient air, fence-line emissions, gasoline stations, automobile emissions (idling and transit through local and commuter traffic patterns), automobile storage in garages, methanol spills and leakage in confined spaces, and evaporation and transfer from a vehicle into the residence. In addition to methanol, some of the microenvironments will accumulate emission byproducts, particularly formaldehyde (Auto/Oil Quality Improvement Research Program 1992).

For the dermal route (Table 1), the concerns are primarily related to direct contact with methanol fuel in occupational environments such as production processes, on-site transfer to tankers, process tank cleaning and repair, and methanol gasoline station activities. Significant dermal contact with the fuel can also be of concern to the general population because of the lack of personal protection and the possibility of having similar types of situations that can lead to contact with the fuel. The primary exposure situations for members of the general population are self-service gasoline stations, clean-up spills such as in the garage, amateur car repair, use as a solvent, and use of personal 1- and 2-gallon methanol gasoline cans. With dermal exposure to methanol-gasoline mixtures there is also concern that the presence of methanol may increase the absorption of gasoline.

Beyond these major routes of exposure, there is also the possibility of incidental or accidental ingestion because of contaminated foods and spills or leaks into water supplies. Deliberate ingestion, a problem even with gasoline, should not be

neglected, even though it is not a problem in environmental contamination.

Research Recommendations. The introduction of methanol as a fuel will increase exposure of the public in many situations. A coordinated plan for assessing and reducing the possibility of methanol exposure should include a number of components.

- *Exposure scenarios and concentrations need to be better characterized.* There is a need to define the situations in which environmental contact with the raw fuel or its combustion products are likely to occur. Probabilistic distributions, extending from low to moderate to high-end exposures, should be developed for the individual compounds and the mixtures of evaporative and combustion emissions. This effort would provide one of the first comprehensive analyses of human contact in various settings before a new fuel is produced for widespread use by the general population. Although under normal operating conditions (see Table 2) only relatively low exposure levels are foreseen, deliberate or adventitiously produced higher levels will probably occur.
- *Emissions and exposure concentration linkages need to be determined.* There is a need to accurately assess emissions of methanol and methanol by-products, particularly formaldehyde (see chapter) and to convert such emissions into human exposure levels in real-life situations. The validity of the models developed and the data obtained should be determined by ambient and personal monitoring.
- *Exposure and dosimetry models are still lacking.* Quantitative models for both exposure and dosimetry can be used in the early stages of an integrated research strategy to help design experiments testing specific hypotheses about methanol toxicity. For example, emission-exposure models, once validated, can be used to predict typical exposure profiles for individuals using methanol as a fuel. Such model simulations should predict exposure profiles for individuals throughout the day—specifically, methanol concentrations in the breathing zone as a function of time. This information can then be used as input for a dosimetry model that is structured to predict blood levels of methanol and formate in blood and target tissue on the basis of physiologically

based pharmacokinetic (PBPK) models. These predictions permit the investigator to design studies that focus on the appropriate animal model, biological effects, and exposure route. The experimental results would then serve to verify or reformulate the dosimetry model.

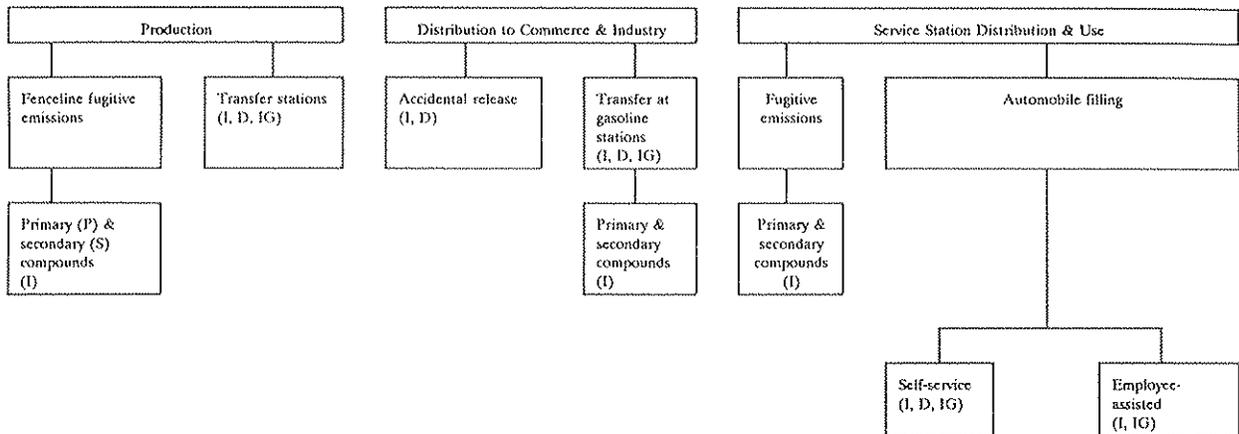
METABOLISM AND PHARMACOKINETICS

Exposure assessment should be carried out in combination with measurements of the amount and form of the agent in target tissue and its disposition over time in the organisms whose exposure is anticipated. For methanol, the primary questions converge on its time course in blood and the fate of its metabolites.

Metabolism

A great deal is known about the metabolism and pharmacokinetics of methanol and formate, the toxic metabolite held responsible for its most damaging effects. These may occur after acute ingestion of, dermal exposure to, or injection of high doses of methanol. Methanol absorbed into blood distributes to tissues in proportion to the fraction of that tissue composed of water. It is completely absorbed from the gastrointestinal tract. The acute damage inflicted by methanol results largely or completely from extreme elevations of blood formate, which leads to a metabolic acidosis and ocular damage. Most of this information comes from a series of elegant studies conducted by Tephly, McMartin, Eells, and associates in the 1970s and 1980s in rodents and nonhuman primates. This information has been presented in review articles (Tephly and McMartin 1984, Kavet and Nauss 1990, Tephly 1991, Eells 1992) and is summarized below under Acute Poisoning from Deliberate or Accidental Exposures.

Methanol is metabolized to formaldehyde, predominantly in the liver, by alcohol dehydrogenase and catalase. Formaldehyde is a reactive compound with a half-life in blood on the order of minutes. It is further metabolized to formic acid by a glutathione-mediated pathway, involving formaldehyde dehydrogenase, which disassociates to formate and hydrogen ions.

Table 1. Distribution of Methanol Fuels and Opportunities for Exposure

I = Inhalation exposure
 D = Dermal exposure
 IG = Ingestion
 S = Secondary compounds
 P = Primary compounds

Table 1. Distribution of Methanol Fuels and Opportunities for Exposure (continued)

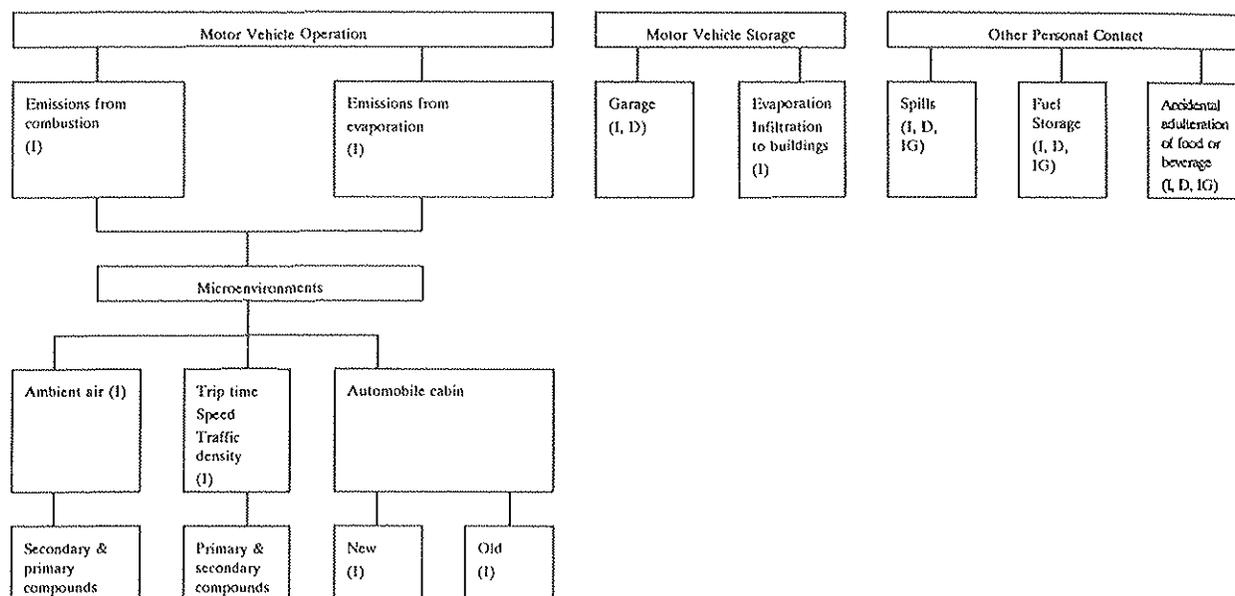


Table 2. Predicted Methanol Exposures and Estimated Body Burden for Selected Situations^a

Scenario	Exposure Concentration ^b	Added Body Burden ^c
Personal garage (hot soak ^d) (resting ventilation X 2)	200 mg/m ³ ; 15 min	0.6 mg/kg ^e
	or 100 mg/m ³ ; 5 min	0.1 mg/kg ^e
Self-service refueling (twice resting ventilation)	50 mg/m ³ ; 4 min	0.04 mg/kg ^e
12-oz diet beverage	555 mg aspartame/L	0.3 mg/kg ^f
Normal dietary intake of aspartame (replacement of sugar with aspartame)		0.3–1.1 mg/kg/day ^f

^a Taken from Kavet and Nauss (1990).

^b 1.3 mg/m³ = 1 ppm.

^c Body burden added onto background exposure of 0.5 mg/kg (of body weight)/day.

^d Evaporation from hot engine at trip end.

^e Assumes all inhaled methanol is absorbed across the respiratory epithelium; most likely only 60% to 70% is absorbed (Sedivec et al. 1981, Gerde and Dahl 1991).

^f Assumes all aspartame-derived methanol crosses the gut mucosa instantaneously.

Formate is then oxidized to carbon dioxide (CO₂). In all species studied, the conversion of formate is achieved via a folate-dependent pathway. Rodents are known to be more efficient in the metabolism of formate to CO₂ than human and nonhuman primates and do not accumulate formate significantly at any methanol doses. In primates, at oral methanol doses greater than 0.2 g/kg of body weight, however, the rate of formate production exceeds that of formate oxidation and formate tends to accumulate (Health Effects Institute 1987). Although methanol can be removed from the systemic circulation by exhalation and urinary elimination, metabolism is the most important mechanism for doing so. Formate itself is not removed by pulmonary exhalation, but by metabolism to CO₂, which is eliminated by the respiratory route; urinary elimination of formate, although measurable, is small.

Unlike primates, lethality in rodents is due to methanol intoxication in the absence of excess formate. Because of such species differences, the disposition of methanol in rodents cannot serve as a model for extrapolation to humans receiving high doses of methanol. However, formate accumulation and the formate-induced toxic symptoms of methanol poisoning can be induced in

rodents with a reduced folate status (Eells et al. 1981, Lee 1989). Deficiencies in hepatic folate or tetrahydrofolate can cause an increase in blood levels of formate and thus increase the likelihood of toxic effects. Nonhuman primates with dietary folate deficiency have also been shown to be more sensitive to methanol poisoning (McMartin et al. 1977). Such findings suggest that humans with a dietary folate deficiency might be more sensitive to methanol exposure. Folate deficiency is common during pregnancy, particularly in women who do not take vitamin supplements, and has been proposed as a risk factor in developmental abnormalities such as spina bifida (MRC Vitamin Study Research Group 1991). Folate deficiency also occurs in chronic alcoholism, as a result of poor diet, certain drug regimens, and is a common complication of diseases of the small intestine, which interfere with the absorption of folate from food.

Background Biological Burdens of Methanol and Formate

Methanol is a natural component of body tissues. The two most prominent sources of background body burdens for methanol and formate are diet

Table 3. Comparison of Methanol Blood Concentrations Following 6 Hours of Exposure in Rats, Monkeys, and Humans

Exposure Concentration (ppm)	Blood Methanol ($\mu\text{g}/\text{mL}$ blood)		
	Rat ^a	Monkey ^a	Human ^b
200	3.1 \pm 0.4	3.9 \pm 1.0	6.97 \pm 1.24
1,200	26.6 \pm 2.0	37.6 \pm 8.5	— ^c
2,000	79.7 \pm 6.1	64.4 \pm 10.7	— ^c

^a mean \pm SEM. Data taken from Horton and coworkers (1992).

^b mean \pm SEM. Data taken from Lee and associates (1992).

^c No experiments conducted at this concentration.

and natural metabolic processes. Methanol is available in the diet from eating fresh fruits and vegetables or from drinking fruit juices. A popular artificial sweetener, aspartame, has become part of many diets. Aspartame hydrolyzes in the gut and 10% by weight is converted to free methanol that is available for absorption (Kavet and Nauss 1990). Methanol is also generated endogenously by the action of a methyltransferase enzyme system. The contributions of diet and metabolism to the body burden of methanol, relative to that arising from projected exposure to inhaled methanol from alternative fuels, must always be kept in context. As demonstrated in Table 2, the contributions of diet and metabolism are of the same order of magnitude as those projected from many exposure scenarios involving inhalation of vaporized methanol-based fuels.

Formate, a toxic metabolite of methanol, is also an endogenous biomolecule. It is a product of the metabolic degradation of several amino acids and is a precursor for a variety of macromolecules. Endogenous or background levels of formate in blood range from 3 to 19 $\mu\text{g}/\text{mL}$ (0.07 to 0.4 mM). Thus, exposure to methanol-derived formate must always be evaluated in the context of normal endogenous levels (see Kavet and Nauss 1990). This issue should also be considered in experimental designs. For example, the diets of subjects in human clinical studies would have to be known and controlled.

Pharmacokinetic Modeling

Horton and associates (1992) conducted experimental studies in nonhuman primates and rats exposed to methanol vapors from 50 to 2,000 ppm for 6 hours. These investigators could not detect elevated levels of formate in blood as a result of the methanol exposures. Experiments conducted in humans exposed in a chamber to 200 ppm (the threshold limit value, TLV) for 6 hours also failed to detect any increase of formate in blood (Lee et al. 1992) and urine (Franzblau et al. 1992). Because environmental levels of methanol stemming from its adoption as a fuel are projected to be considerably lower than the current TLV in most scenarios, formate should not present a hazard under these circumstances. Blood methanol concentrations for these experiments are compared in Table 3.

On the basis of experimental work conducted by themselves and others, Horton and associates (1992) developed a physiologically based pharmacokinetic model for methanol disposition in rats, humans, and nonhuman primates. The model was used to predict the atmospheric methanol concentration range over which laboratory animal species might exhibit quantitative similarities to humans in methanol metabolism. According to the model, below 1,200 ppm all three species should exhibit similar end-of-exposure blood methanol concentrations and linear relationships between atmospheric and blood methanol concentrations. At higher atmospheric concentrations, the predicted blood concentration would no longer be linearly related to the inhaled concentration, which suggests that methanol blood levels, rather than

exposure concentration, should be the basis for interpreting data from high-dose studies. These findings also show the importance of using experimental concentrations closer to real-world levels if results are to be extrapolated to human scenarios.

Recent experimental evidence showing that in utero exposure to high concentrations of methanol vapors produces exencephaly in mice, but not in rats (see below under Developmental Effects), indicates that the pharmacokinetics of methanol and formate should be further investigated in pregnant animal models to understand the possible roles of these chemicals. PBPK modeling provides a quantitative approach to studying exposure-dosimetry relationships and was used previously to investigate the pharmacokinetics of a few compounds during pregnancy in rodents. One model that provides similarities to methanol is that developed for methoxyethanol and its major metabolite, methoxyacetic acid (Clarke et al. 1993). These models can be used to study maternal/fetal dosimetry at various stages of gestation. Research needs evolving from such questions are discussed more extensively in the section on developmental effects.

Research Recommendations. In most ordinary environments, projected exposures to methanol fuel emissions are not expected to increase formate levels in the blood of normal individuals; however, there is very limited information to determine whether subgroups of the general population may be more sensitive to methanol exposure. In addition, there are no data available to determine whether formate may accumulate in the target tissues of humans who are exposed repeatedly and at sufficiently short intervals to methanol vapors. A number of questions, therefore, remain about the impact of methanol emissions on methanol and formate accumulation in the general population.

- *The disposition of methanol and formate in blood and target tissues and in the maternal/fetal unit needs to be determined in laboratory animals and extended to normal humans as well as those with a compromised dietary status.* The range of exposure should include those exposures projected to occur under conditions of general use and in occupational settings. These are crucial data for risk assessment.

- *Because most of the available data on toxicity are based on high acute doses, it remains to be determined whether blood concentrations of methanol and formate are acceptable surrogates for target tissue concentrations at low levels of exposure and whether dosimetry is altered by folate status.* Resolution of this question requires information about metabolism in target tissues.

HEALTH ISSUES

No simple answers can be given to questions framed in terms of "How much is safe?" Methanol exemplifies those agents whose spectrum of toxicity changes with dose. The transition is particularly troublesome with methanol because the mechanisms of acute, high-dose effects are likely to be different in character from those at low and moderate exposure levels. Exposures during early development arouse further questions because the expression of toxicity may be delayed until the organism has attained a sufficient degree of maturity. Although the issues pertaining to effects at high and low exposures are separated below for convenience, they remain intertwined; information from one of these models may offer useful guides to the others.

EFFECTS OF OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES

This section of the document examines current knowledge of the health effects of methanol in both humans and animals that may be of concern at occupational and environmental exposure levels and indicates the most apparent gaps in data. The list of research needs follows from this evaluation.

Contemplated exposures of the general public arising from the adoption of methanol as a fuel for motor vehicles ordinarily would fall within a range low enough to preclude marked elevations in blood formate levels. Under these circumstances, the presumed health risks would typically stem from methanol exposure itself. Moderate elevations in methanol concentrations or transient high levels sufficient to raise formate levels significantly above baseline pose another set of questions alluded to above.

Neurobehavioral Toxicity

Human Studies Most of our current knowledge of methanol toxicity in humans comes from accidents stemming from the adulteration of alcoholic beverages. Although such data may be of limited use in setting workplace and community standards, they provide clues to mechanisms and sites of action and guides to indices that may serve as endpoints in formal studies. For example, the latent period following acute exposure to high doses of methanol, which may culminate in metabolic acidosis and visual system damage, usually triggers nonspecific symptoms such as headache, dizziness, and blurred vision. These early manifestations correspond to signs reported in subacute or chronic situations that do not produce the severe reactions associated with acute, high-dose poisoning.

Workplace exposures are the source of most of our information about methanol neurotoxicity below catastrophic levels. Individual case studies in the literature from 1900 to 1960 describe occupational poisonings resulting from inhalation exposure to methanol (Buller and Wood 1904, Humperdinck 1941, Browning 1965). Although the details of exposure (duration and concentration) are usually missing, the effects of prolonged exposure are qualitatively very similar to those reported for acute cases, ranging from nausea and dizziness to blurred vision and temporary or permanent blindness. On the basis of the accumulated evidence, the National Institute of Occupational Safety and Health (NIOSH) (1976) suggested that repeated exposure to 1,200 to 8,300 ppm (1,572 to 10,873 mg/m³) can lead to impaired vision.

The most systematic observations in occupational settings were secured from workers operating duplicating ("ditto") machines, now largely supplanted by Xerox technology. Vapor concentrations from 365 to 3,080 ppm (478 to 4,034 mg/m³) have been claimed to induce headache, dizziness, nausea, and blurred vision (Kingsley and Hirsch 1954, Frederick et al. 1984). Similar complaints are common in workers exposed to volatile organic solvents (Anger 1990). Although dose-effect relationships are not established for chronic methanol exposure, this literature suggests that prolonged exposures to concentrations of methanol above 200 ppm (or 260 mg/m³) may engender signs of visual and central

nervous system toxicity. Such reports indicate that acute and chronic effects of methanol may share a common mechanistic basis.

The only experimental attempt so far to evaluate acute neurobehavioral effects was undertaken by Cook and associates (1991). In this pilot study, volunteers were exposed to a mean concentration of 192 ppm (close to the TLV of 200 ppm; American Conference of Governmental Industrial Hygienists 1991), and their performance was assessed on a battery of neurobehavioral tests. These included electrophysiologic measures such as evoked potentials, cognitive measures such as memory and coding functions, motor coordination measures such as hand steadiness, reaction time, and measures of subjective variables. The subjects were exposed for 75 minutes on each of four occasions, two of which included methanol vapor delivered to the exposure chamber. Changes in a few of the measures, such as memory performance, approached statistical significance. Scores on a scale of fatigue showed a significant association with methanol exposure. Although none of the changes appeared to be of notable magnitude, the authors pointed out the small size of their sample, the restriction to a single exposure concentration, and the difficulty of maintaining double-blind conditions, circumstances that together attenuate the possibility of finding significant responses. Moreover, limiting the subject sample to healthy young men restrains the scope of extrapolation, particularly to what would be considered susceptible populations.

Animal Studies Few data are available about neurotoxic responses other than from studies designed to mimic severe human intoxications. Acute studies with neurobehavioral endpoints are lacking except for the experiment by Youssef and associates (1993, in press), which yielded a linear fit to the relationship between reduced running-wheel performance in rats and oral methanol dose. The rats were given doses of 1, 2, or 3 g/kg by gavage before an experimental session. A study by Mullenix and associates (1991) found that rats treated orally with 0.5 or 1 g/kg aspartame (yielding methanol doses of 0.05 and 0.1 mg/kg, respectively) did not exhibit modifications in spontaneous behavior.

In one subchronic inhalation study, cynomolgus monkeys were exposed for four weeks (6 hours/day, 5 days/week) to 500 to 1,500 ppm

methanol vapors (Andrews et al. 1987). No clinical signs of toxicity were observed in these animals during the study, and no histologic alterations were found in tissue sections prepared from lung, liver, retina, and optic nerve at the termination of the study.

Long-term studies of aspartame toxicity in monkeys have indirectly provided information on chronic methanol exposure. Aspartame hydrolyzes in the gut to aspartate, phenylalanine, and methanol, with methanol accounting for 10% of the molecular weight of aspartame. One study (Suomi 1984) examined the consequences of aspartame exposure of newborn *Macaca speciosa* for 9 months, but the calculated methanol doses (100 to 270 mg/kg/day¹) failed to elicit any detectable changes in behavioral and neurologic parameters, growth patterns, hematology, or serum chemistry effects. Histologic evaluations of liver, kidney, brain, retina, and optic nerve were not conducted. The prenatal period, however, may be much more critical in primates, as in the case of ethanol (Clarren et al, 1987)

Research conducted in Japan (New Energy Development Organization 1987) suggested that chronic exposure of primates to relatively low levels of methanol (100 and 1,000 ppm, 20 hours/day) produced a neuropathologic abnormality labeled as "reactive astrocytes," which might be interpreted as a hyperplastic response of astroglia, and degeneration of the inside nucleus of the thalamus. No degeneration of the optic nerve was observed at any concentration. However, this study has not been fully reported, and these results remain to be clarified.

Research Recommendations.

- *Human experimental data on acute effects are lacking.* Although the study by Cook and associates (1991) was a useful beginning for assessing human neurotoxic responses, additional experimental exposures, particularly to a range of vapor levels and for longer periods, are required. Moreover, subjects other than healthy young men should be studied. The animal literature, for example, suggests that older organisms are more susceptible than young adults to methanol toxicity, according to lethality indices (Kimura et al. 1971). In addition, more definitive assessments of visual and motor function than those reported by Cook and associates should be conducted.
- *Flash-evoked potentials and critical flicker fusion measures are relatively crude, nonspecific, and even misleading indices of visual function.* Limb steadiness should be characterized by advanced techniques such as power spectral analysis (see Newland and Weiss 1991), and more advanced measures of coordination and other aspects of fine motor function also need to be implemented in comprehensive studies in humans and animals.
- *A system for monitoring the effects of chronic exposures should be implemented.* There is also a dearth of information about the effects of chronic exposures to methanol. Suitable populations should be identified (including females of childbearing age, whose offspring may be at risk based on studies of workplace exposure to solvents and studies of methanol exposure in pregnant laboratory animals; see below under Developmental Effects) and, if possible, followed for extended times by periodically administering neurobehavioral tests.
- *An extensive program of research on laboratory animals is required.* Only sparse information about neurobehavioral toxicity in animals is available, especially from experiments incorporating advanced measurement techniques. The main gaps exist in studies at low and moderate exposure levels, in assessments of chronic exposures, and in experiments with possibly susceptible populations such as old animals, neonates, and animals with compromised nutritional status such as folate deficiency.
- *Mechanisms of neurotoxicity need to be elucidated.* Damage to the putamen has been described as an aftermath of severe, acute intoxication. Although dopamine is considered the dominant neurotransmitter in striatum, there are many other candidates for neurotoxic action besides dopamine-containing and dopamine-responsive structures. Animal work is desirable to amplify this information. Imaging studies (positron emission tomography and magnetic resonance imaging) in victims of intoxication or in exposed workers could prove valuable, especially because of the predominant concern of chronicity.
- *Folate deficiency itself poses important questions.* The neurobehavioral concomitants of combined folate deprivation and methanol exposure might

provide useful data applicable to sensitive subpopulations. Furthermore, species other than rodents have not been examined adequately. Avian species, for example, are not only sensitive to folate deficiency, but, unlike rodents, are primarily visual animals and are often subjects of choice in visual system research pursuing questions about color vision and acuity. However, before such studies are conducted, it would be important to know more about the metabolism and pharmacokinetics of methanol and formate in these species to ensure that they would be appropriate models for the neurotoxicity experiments.

- *The products of methanol combustion, particularly formaldehyde, may also interfere with performance as measured behaviorally.* Some reports associate formaldehyde exposure with behavioral effects (see chapter on formaldehyde). Another irritant, ozone, demonstrates such properties (Weiss 1988). In addition, methanol will most likely be available in combination with gasoline (M85), which contains several neurotoxic compounds. One of these compounds, toluene, has been the focus of many investigations and may degrade behavioral performance even at the current TLV of 100 ppm (Echeverria et al. 1991). Thus, the potential neurobehavioral effects of exposures to mixtures need to be investigated.

The Visual System and Ocular Toxicity

The primary adverse outcome of methanol intoxication in humans and other primate species is damage to the visual system. Except under the special circumstances mentioned above under Metabolism and Pharmacokinetics, typical laboratory species such as rodents are extremely resistant to such effects. Because visual system toxicity is a major concern at high methanol doses, more subtle disturbances of visual function might also provide useful endpoints for assessing the health risks of chronic or repeated exposure to low and moderate levels of methanol.

Human Poisoning The minimum dose causing permanent visual defects is unknown, although blindness has been reported after ingestion of as little as 4 mL of methanol (Bennett et al. 1953, Tong 1982). Visual disturbances generally develop between 18 and 48 hours after ingestion and range

from mild photophobia and misty or blurred vision to markedly reduced visual acuity, altered visual fields (reduction or loss of central vision), and complete blindness. The functional and morphologic bases of the visual toxicity remain controversial. Both the retina and optic nerve have been postulated to be the primary site of the toxic lesion in methanol poisoning on the basis of ophthalmoscopic and histologic evidence.

Electrophysiologic and morphologic evidence for direct retinal toxicity has been reported in a case of fatal human methanol poisoning (Eells 1992). Histopathologic studies disclosed mitochondrial swelling and disruption in the photoreceptor cells and pigment epithelium of the retina and axonal swelling and mitochondrial disruption in the retrolaminar optic nerve (see Sharpe et al. 1982).

Nonhuman Primates Gilger and Potts (1955) were the first to document alterations in pupillary light response, and retinal and optic disc edema, in methanol-intoxicated (2 to 3 g/kg) monkeys (*M. mulatta*) that were similar to the signs observed in human poisonings. Electroretinographic measures also revealed a reduction in b-wave amplitude indicative of retinal toxicity and were confirmed by Ingemansson (1983). Studies by Martin-Amat and associates (1977) have yielded a reproducible nonhuman primate model of methanol-induced visual toxicity using an attenuated, repeated administration of methanol. The major features of this animal model included formate accumulation, metabolic acidosis, and pathologic alterations similar to those observed in human cases of methanol poisoning. Martin-Amat and associates (1978) have also demonstrated ocular toxicity, similar to that produced by methanol, in rhesus monkeys infused with buffered formate at physiologic pH, indicating that formate-induced visual toxicity is independent of acidosis. Permanent visual damage in methanol- and formate-intoxicated monkeys (Hayreh et al. 1980) and in methanol-poisoned humans (Jacobsen and McMartin 1986) is associated with prolonged exposures (usually of more than 20 hours) to blood formate concentrations in excess of 7 mM.

The Folate-Deficient Rat Model Studies also demonstrated methanol-induced ocular toxicity in rats that were folate-deficient (Lee 1989) and tetrahydrofolate-deficient (Eells 1991, Murray et al. 1991). Methanol-intoxicated (4 g/kg plus

supplemental 2 g/kg doses at 12-hour intervals), tetrahydrofolate-deficient rats developed signs of formic acidemia, metabolic acidosis, and visual toxicity within 36 hours of methanol administration analogous to the human methanol poisoning syndrome. Visual dysfunction was measured as reductions in the flash-evoked cortical potential and electroretinogram (reduction in the b-wave), which occurred coincident with blood formate accumulation. Alterations in the electroretinogram occurred at formate concentrations lower than those associated with other visual changes and provide functional evidence of formate-induced retinal toxicity. Histopathology revealed mitochondrial disruption and vacuolation in the retinal pigment epithelium, photoreceptor inner segments, and optic nerve. Lee (1989) also has reported reductions in the b-wave of the electroretinogram (ERG) in folate-deficient rats (folate-deficient diet plus succinylsulfathiazole) given a single oral dose of methanol (3.5 mg/kg) or exposed to 800 ppm (1,048 mg/m³) of methanol vapor for 90 days.

In methanol-intoxicated, tetrahydrofolate-deficient rats, relatively brief exposures to formate produce reversible retinotoxic actions detectable by electroretinography. Furthermore, these ERG alterations occur at formate concentrations lower than those required to affect the flash-evoked cortical potential and lower than those associated with retinal and optic disc edema in humans and monkeys (Martin-Amat et al. 1977, Hayreh et al. 1980, McMartin et al. 1980). ERG alterations in human methanol poisoning also have been observed without evidence of other clinical signs of retinal toxicity (Eells 1992). The ERG may therefore furnish a noninvasive diagnostic tool with which to assess the degree of retinal toxicity in methanol poisoning.

Mechanism of Formate Toxicity The mechanism by which formate produces retinal and optic nerve toxicity is not known. One explanation holds that formate disrupts cellular energy production by inhibiting the activity of cytochrome oxidase (Nicholls 1976, Martin-Amat et al. 1977, Eells 1992). Selective inhibition of mitochondrial function in the retina and optic nerve is also consistent with both the functional and morphologic findings in methanol intoxication. However, further investigations are needed to test this hypothesis.

Research Recommendations.

- *Dose-effect and time-course relationships between blood and tissue formate concentrations and the onset and development of visual toxicity have not been established.* The relationship between concentration and duration of formate in the blood and signs of visual system toxicity is unclear. Measures of visual system function, including the electroretinogram, visual-evoked potentials, and visual discrimination performance may provide sensitive indicators of visual system dysfunction. The degree of reversibility of functional and structural changes in the visual system is also unknown, as is the possibility of cumulative effects from repeated transient elevations of formate levels.
- *It is unclear whether the retina and the optic nerve can metabolize methanol.* If metabolic systems are present in retina or optic nerve that convert methanol to formaldehyde and formate, toxic metabolites may be produced locally. Such tissue-specific differences in the generation of formaldehyde and formate may contribute to the observed sensitivity of the retina and optic nerve. Studies on the metabolism of formate to CO₂ in retina and optic nerve will determine the capacity of these tissues to detoxify formate. The presence and specific activity of methanol-metabolizing systems in ocular tissues could have important implications for evaluating the potential consequences of chronic or repeated low-level exposure to methanol.
- *The sensitivity of the visual system to acute and chronic methanol exposure at low or moderate levels is uncertain.* One important area of uncertainty is the role of formate under such conditions. Appropriate studies would provide information relevant to human risk parameters by exploring a range of dose-effect functions. Appropriate animal models and tests would have to be used. As mentioned above, avian species are often subjects of choice in visual system research on color vision and acuity. They may prove to be useful for this type of research. Human studies should include sensitive tests directed toward central visual function, such as color discrimination and spatial contrast sensitivity, which are more pertinent than flash-evoked potentials and critical flicker fusion measures because

impaired central visual function is characteristic after methanol poisoning.

- *The full spectrum of nutritional, metabolic, and age-related factors that may influence susceptibility to methanol-induced visual toxicity is obscure.* As noted above, formate oxidation (detoxification) to CO₂ depends on the functional integrity of the folate-dependent pathway. Furthermore, animal studies have shown that treatments which reduce hepatic tetrahydrofolate concentrations decrease formate metabolism and enhance the toxic response to methanol. Thus, it is likely that folate or vitamin B₁₂ deficiency, which itself produces central retinal lesions, influences susceptibility to methanol toxicity. The relationship between these deficiencies and possibly other deficiency states and susceptibility to chronic methanol exposure is equivocal. Diseases of the visual system, which are much more common in advanced age, may also be predisposing factors for methanol toxicity. Poor nutritional status is common as well in old age. Blood and tissue formate assays and functional assessment of visual toxicity should be performed in studies designed to answer these questions.

Neuroendocrine Effects

Concerns over potential adverse effects on the male reproductive system have been raised because of reports that 200 ppm methanol, the current TLV, lowered levels of blood testosterone in rats (Cameron et al. 1984, 1985). Serum testosterone levels were reported to be reduced after a single exposure to 200 ppm for 6 hours, after exposure to 200 ppm for 2 and 6 weeks, and after exposure to 2,000 ppm for 6 weeks, but not after exposure to 10,000 for up to 6 weeks, indicating that the observations were not dose-related. The repeated exposures also led to an increase in the concentration of luteinizing and follicle-stimulating hormones in the serum, suggesting that compensatory changes in pituitary hormone secretion occur after repeated exposure.

Other investigators, however, were unable to confirm those initial observations (Lee et al. 1991, Cooper et al. 1992). In addition, Cooper and associates (1992) found that, although acute methanol exposure can cause significant alterations in serum hormone levels, the direction and

magnitude of the changes were inconsistent and depended on whether or not the animals had been acclimated to the test situation.

Lee and associates (1991) also examined the potential of methanol to modify testes morphology in rats of different ages. Rats were placed on folate-reduced (FR) diets also containing 1% succinylsulfathiazole, often used in nutrition studies to minimize intestinal synthesis of folate. The investigators observed a greater incidence of testicular degeneration (subcapsular presence of vacuoles) in 18-month-old FR rats. Neither 10-month-old FR rats exposed to 800 ppm methanol, 20 hours/day, for 13 consecutive weeks, nor rats fed a folate-sufficient diet exhibited such signs. These findings suggest that methanol may have the potential to accelerate normal age-related degeneration of the testes.

Research Recommendations.

- *Can methanol accelerate age-related degeneration of the testes?* This is an important question because even a minor acceleration has broad public health implications. If the initial observations are verified, the mechanisms of toxicity can be explored. Combinations of aging and nutritional status may converge to produce particularly susceptible populations, as noted in the discussion of visual system toxicity. A related issue may be posed as well. Data derived from studies of male animals exposed to lead and ethanol suggest that these agents induce effects on male reproductive function that are subsequently reflected in the neurobehavioral development of the offspring.
- *Does methanol affect testosterone levels during fetal development?* As noted in the section below, developmental toxicity is a major concern of methanol exposure. Beyond the question of more direct and conventional endpoints, subtle consequences arising from modified testosterone levels during early brain development may pose an even more confusing issue. Androgens during the prenatal period and part of the early postnatal period can determine the expression of behavioral characteristics (Kimura 1992). If these levels are modified by methanol exposure, sex-specific behavior patterns, ranging from reproduction to problem-solving, might also be modified.

Carcinogenesis

Limited data are available about effects of methanol on carcinogenesis. The only cancer bioassay has been conducted by New Energy Development Organization (1987). In this study, rats were exposed to methanol vapors at concentrations of 0, 10, 100, and 1,000 ppm for 19 hours/day for 24 months, and mice were exposed to the same methanol concentrations for 18 months. The exposures did not affect body weight or survival rates in either species. Pathologic examination revealed a slightly higher incidence of tumors in the lung (papillary adenoma) in male rats in the 10 and 1,000 ppm groups, and of tumors of the adrenal gland (chromaffinoma) in female rats in the 1,000 ppm group. No significant histopathologic changes were observed in mice. These results suggest that methanol is not carcinogenic in mice, but raise the possibility that it may be a very weak carcinogen in rats. These studies have not been published in the peer-reviewed literature.

McDonald and associates (1992) conducted an *in vivo* study to determine whether exposure to methanol induces cytochrome P-450 and thus potentiates the toxicity of other compounds that are activated by this system. The experiments consisted of exposures of rats ranging from 1,000 to 10,000 ppm methanol vapors for 6 hours followed by exposure to carbon tetrachloride (CCl₄). The results indicated that only exposures to methanol concentrations of 5,000 ppm or greater induced specific forms of the P-450 systems and increased CCl₄ toxicity in a dose-dependent manner when CCl₄ was administered on the first day after methanol exposure. Another study found that methanol caused the formation of a potent mutagenic metabolite of a carcinogenic aromatic amine in the *Salmonella* assay in the presence of S9 (Cunningham et al. 1990). Assuming these findings can be confirmed, the potential for methanol to activate toxic compounds should be considered when assessing the carcinogenicity of methanol.

Research Recommendations. The issue of whether methanol may be a carcinogen is still unresolved, but the limited information available indicates that carcinogenesis does not appear to be a major health issue. A remaining concern is the potential for methanol indirectly to activate toxic compounds, which may themselves be

carcinogenic. Further research is needed to determine whether relevant levels of methanol indeed induce P-450 isozymes in humans.

Developmental effects

The developing organism exhibits an enhanced vulnerability to many chemical agents. The thalidomide tragedy engendered comprehensive requirements for premarket drug testing designed to prevent the occurrence of repetitions. The fetal brain is recognized as the target of many neurotoxicants, such as lead and methylmercury. Dismorphology, as well as mental retardation, is an outcome of maternal abuse of ethanol. Retinoic acid treatment of severe acne has been indicted for the same reason. Because of this history, it is reasonable to be especially alert to the potential developmental toxicity of methanol, which, unlike drugs, would be distributed without any medical monitoring. The appropriate criteria of toxicity range from birth defects to motor behavior problems and attention deficit disorders.

Developmental Toxicity of Methanol in Rats

Nelson and coworkers (1985) exposed pregnant rats to 0, 5,000, 10,000, or 20,000 ppm methanol for 7 hours/day on gestation days 1 to 19 (7 to 15 for 20,000 ppm). The highest dose produced slight maternal toxicity and a variety of congenital malformations and anomalies, including defects of the cardiovascular, urinary, and central nervous systems. A low incidence of similar anomalies was seen at 10,000 ppm, but was not significantly different from that in control rats. Exposure of dams to 10,000 ppm produced no maternal toxicity, but resulted in a significant reduction in fetal weight at term. Thus, in this study, the NOAEL for maternal toxicity was 10,000 ppm and for developmental toxicity was 5,000 ppm. Similar exposure concentrations of ethanol engendered no appreciable developmental toxicity.

Altered behavioral development of offspring following exposure of pregnant Long-Evans rats via the drinking water was reported by Infurna and Weiss (1986). These investigators administered 2% methanol in the drinking water of pregnant Long-Evans rats on gestation days 15 to 17 or 17 to 19. Daily methanol consumption averaged 2.5 g/kg over the 3-day periods. The responses of suckling (postnatal day 1) and nest-seeking (postnatal day 10) were examined in offspring. No maternal

toxicity or effects on litter size, birth weight, or neonatal mortality were seen, and postnatal growth was unaffected. However, both suckling behavior and nest-seeking were adversely affected in pups of methanol-exposed dams at either gestational period, indicating that maternal methanol exposure caused behavioral abnormalities that were not accompanied by maternal toxicity.

Stanton and associates (1991) exposed pregnant rats to 15,000 ppm inhaled methanol for 7 hours/day on days 7 to 19 of gestation. Peak maternal blood methanol levels were about 3 mg/mL throughout the exposure period. Pups were tested with a behavioral battery through postnatal day 73, and no effects were noted.

Developmental Toxicity of Methanol in Mice

Rogers and associates (1993) have conducted studies in pregnant CD-1 mice to examine the sensitivity of this species to the developmental toxicity of inhaled methanol. Pregnant mice were exposed to 1,000, 2,000, 5,000, 7,500, 10,000, or 15,000 ppm methanol for 7 hours/day on days 6 to 15 of gestation. Sham-exposed controls were exposed to filtered air, and other control groups were left in their home cages either unhandled or food-deprived for 7 hours/day to match the food deprivation experienced by the exposed mice. Blood methanol concentrations were determined on gestation days 6, 10, and 15. Significant increases in the incidence of exencephaly and cleft palate were observed at 5,000 ppm and above, increased embryo or fetal death at 7,500 ppm and above (including an increasing incidence of full-litter resorptions), and reduced fetal weight at 10,000 ppm and above. A concentration-related increase in cervical ribs or ossification sites lateral to the seventh cervical vertebra was significant at 2,000 ppm and above. Thus, the NOAEL for developmental toxicity in this study was 1,000 ppm.

A log-logistic dose-response model was applied to the incidence data for exencephaly, cleft palate, resorption, and cervical rib, and maximum likelihood estimates (MLEs) and benchmark dosages (BDs, the lower 95% confidence interval of the MLEs) corresponding to 1% and 5% added risk above background were calculated. The MLE for 5% added combined risk of having either exencephaly or cleft palate or being resorbed was 3,667 ppm, and the corresponding BD was 3,078 ppm. For cervical rib, the 5% added risk values for the MLE and BD were 824 and 305 ppm,

respectively. The BDs for 1% added risk were 1,915 ppm for exencephaly, cleft palate, or resorption, and 58 ppm for cervical rib.

Litters of pregnant mice gavaged orally with methanol at 4 g/kg were examined for resorption, external defects (including cleft palate), and fetal weight. Incidences of adverse effects on these endpoints were similar to those in the 10,000 ppm methanol inhalation exposure group.

Parallels Between Methanol and Ethanol

The developmental toxicity of ethanol has probably been studied as extensively as that of any other chemical, but its mechanisms of action at the molecular-biochemical level are still poorly understood. Some of the proposed mechanisms, however, would be expected to hold for methanol as well. Among these are peroxidative damage due to free radical production, cell membrane effects, and interference with the metabolism of retinoic acid (via alcohol dehydrogenase) or folate (via tetrahydrofolate reductase). In another point of correspondence between the two alcohols, the methanol gavage studies in mice, noted above, used two doses of 2 g/kg each to produce terata, which is similar to the intraperitoneal dose used by Sulik and associates (1981) in their mouse model of fetal alcohol syndrome (2.9 g/kg). Because of these parallels, it would not be surprising if others were observed.

Research Recommendations.

- *Animal models of methanol-induced developmental effects need to be better characterized.* Inhaled methanol produces birth defects in the mouse at levels lower than those that are effective in the rat. However, there are few data on methanol pharmacokinetics and metabolism in the mouse, or on these parameters during pregnancy for any species. Therefore, it is critical to describe the uptake and distribution of inhaled methanol in the pregnant mouse as well as in the rat to determine whether species differences in sensitivity to inhaled methanol are due primarily to pharmacokinetic differences. These studies should include measurement of formate to see whether formate accumulates following developmentally toxic exposures and of folate levels. Data on the extent of formate accumulation will also be important in identifying whether methanol or formate is the

- proximate developmental toxicant in the animal models.
- *Initial findings of methanol-induced effects need to be confirmed and extended for hazard identification.* Further studies on the developmental effects of methanol exposure are important, including postnatal manifestations of prenatal, perinatal, or neonatal exposure. Postnatal survival, growth and neurobehavioral development have not been adequately studied following prenatal and neonatal exposure to methanol and should be examined in rodent as well as primate species.
 - *The interaction of methanol exposure and folate metabolism needs to be elucidated.* Pregnant women are known to be a subpopulation with a relatively high incidence of folate deficiency, and folate deficiency has been associated with neural tube defects in humans (Milunsky et al 1989, MRC Vitamin Study Research Group 1991). A recent report indicates far greater folate requirements during pregnancy than previously believed (McPartlin et al. 1993). Since methanol is metabolized via the folate pathway, it is critical that the relationship between methanol metabolism and folate metabolism be elucidated. Experiments should investigate whether folate deficiency increases susceptibility to methanol and, conversely, whether methanol exposure adversely impacts folate metabolism, leading to induced or enhanced folate deficiency.
 - *Mechanism of action of methanol and its metabolites in inducing developmental effects is not understood.* Virtually nothing is known about the mechanism of action by which the developmental toxicity of methanol (and/or formate) is induced. One starting point is to take advantage of the extensive literature on the developmental toxicity of ethanol. There are mouse models for fetal alcohol syndrome, and the pathogenesis of malformations has been described. Studies on the pathogenesis of methanol-induced malformations should be carried out to determine similarities to ethanol. The biochemical and cellular mechanisms of the developmental toxicity of ethanol are also poorly understood; several viable hypotheses have been proposed that could be examined for methanol as well. Induced folate deficiency as a mechanism of methanol developmental toxicity should also be studied.

ACUTE POISONING FROM DELIBERATE AND ACCIDENTAL EXPOSURES

As mentioned earlier, the introduction of methanol fuel will pose the risk of accidental spills and abuse. This section discusses the health effects of concern and research needs for exposure levels much higher than those resulting from the normal use of methanol fuel.

Most information about methanol toxicity in humans comes from human episodes of poisoning and acute, high-dose experiments with animals. Although such data are of limited use in establishing risk estimates for exposures arising from controlled, conventional uses of alternative fuels, they do provide a helpful set of perspectives. Moreover, if experience with gasoline provides any guidance, deliberate ingestion, especially by young children, is likely to occur. More than 13,000 cases of acute gasoline exposure were recorded by the American Association of Poison Control Centers in 1987 (Litovitz 1988).

The minimum lethal dose of methanol in the absence of medical treatment is between 0.3 and 1 g/kg (Roe 1982, Health Effects Institute 1987). The clinical literature substantiates wide individual variation to the lethal and sublethal effects of methanol, with fatalities reported after ingestion of 15 to 30 mL of 40% methanol and survival with aggressive medical treatment after ingestion of 500 to 600 mL. Two important determinants of human susceptibility to methanol toxicity appear to be (1) concurrent ingestion of ethanol, which slows the entrance of methanol into the metabolic pathway, and (2) hepatic folate status which governs the rate of formate detoxification. In rats, LD₅₀ values for combinations of ethanol and methanol are not fully congruent with what would be expected on the basis of clinical applications in human poisoning (Youssef et al 1992). In this instance, lethality is due to the combined toxicity of both alcohols; formate is not the culprit in rodents.

In addition to the level of folate or folate metabolites, genetic variations may be involved in determining individual sensitivity; for example, genetic variations in both alcohol (ADH) and aldehyde dehydrogenase, which occur in certain ethnic groups with much higher incidence than in others and may be an important determinant of alcoholism (Agarwal and Goedde 1987, Crabb et al. 1989). At least four different classes of ADH

isoenzymes have been identified (Bosron and Li 1986).

Acute methanol toxicity follows a well-defined pattern. An initial transient, mild central nervous system depression similar to that produced by ethanol is followed by an asymptomatic latent period lasting generally 18 to 24 hours. This latent period is followed by symptoms including headache, vertigo, nausea, vomiting, and intense abdominal pain. Visual disturbances and increased depth and rate of respiration occur concomitant with the development of formic acidemia and metabolic acidosis. In severe poisoning, the patient becomes cyanotic and comatose, and death occurs by respiratory arrest followed by cardiac arrest (Roe 1955, Tephly and McMartin 1984, Health Effects Institute 1987). Although the visual system appears to be the primary target of methanol damage, studies of methanol poisoning cases indicate that certain areas of the brain, primarily the basal ganglia, incur pathologic abnormalities (Aquilonius et al. 1980, Koopman et al. 1988, LeWitt and Martin 1988).

Animal Studies: The Role of Folate-Dependent Formate Oxidation

The pursuit of dose-effect relationships and toxic mechanisms of methanol has been hampered by the lack of an appropriate animal model. This situation is not unique to methanol; nevertheless, the lack of such a model delayed until the 1970s, as described above, the ability to grasp the steps in methanol metabolism that make it such a potent hazard. The pivotal finding, contributed by Tephly and his coworkers, demonstrated that nonhuman primates offered the best animal model for studying human poisoning.

The minimal dose of methanol required to induce symptoms of toxicity is greater in nonhuman primates (3 g/kg; Roe 1982) than in humans (1 g/kg; Roe 1982), yet nonhuman primates exhibit equivalent signs of poisoning: metabolic acidemia and visual disturbances. For this reason, their response to lower exposure levels, both single and repeated, would be important to risk assessment.

The lethal dose of methanol for rodents is 6 to 10 times the lethal human dose of methanol (Gilger and Potts 1955, Tephly and McMartin 1984, Health Effects Institute 1987). Furthermore, the symptoms of acute poisoning in non-primates

are manifested exclusively as central nervous system depression such as those observed with other alcohols (Gilger and Potts 1955). Despite the administration of lethal doses of methanol, rodent species do not develop the formic acidemia, metabolic acidosis, or visual impairment characteristic of human methanol poisoning, making them an inappropriate model for extrapolation to acute human poisoning (Gilger and Potts 1955, Roe 1982, Tephly and McMartin 1984).

The susceptibility of various species to methanol toxicity is inversely related to their capacity for tetrahydrofolate-dependent formate oxidation to CO₂. In rats, folate deficiency induced by a folate-deficient diet or selective reduction of tetrahydrofolate by treatment with nitrous oxide results in formic acidemia, metabolic acidosis, and ocular toxicity (see above) after methanol administration (Palese and Tephly 1975, Makar and Tephly 1976, Eells et al. 1991).

Research Recommendations. The toxic effects of methanol are fairly well documented and understood. If methanol fuel were to be introduced on a large scale, the prevention of such effects should be of the highest priority. Further research in this area should focus on better understanding the mechanism of methanol and formate damage and the underlying factors that determine individual sensitivity to methanol.

- *The mechanism of methanol-induced visual system toxicity and neuropathology needs to be clarified.* The mechanism by which formate, the presumed toxicant, produces retinal and optic nerve toxicity is not known. Such information is essential for a complete understanding of the pathogenesis of methanol neurotoxicity and may facilitate the development of more effective treatments. In addition, tissues and organs that are apparently unaffected in methanol poisoning are also dependent on mitochondrial function.
- *The reason for the wide individual variation in the effects of methanol needs to be understood.* Identifying the causes of selective vulnerability to methanol might help to identify individuals at risk of methanol exposure. These studies will require the proper animal models. The folate-reduced (FR) or tetrahydrofolate-reduced rat could be a suitable animal model and could contribute information to the understanding of

the relationship between FR rats and humans in sensitivity to methanol and the effects of prolonged folate deficiency on biochemical and physiologic function. In addition, the relationship between genotypes for the key enzymes involved in methanol metabolism and methanol and formate blood level should be investigated.

PRIORITIZATION OF RESEARCH RECOMMENDATIONS

Quantitative relationships linking methanol emissions, exposure, tissue dose, and effects are necessary to calculate risk estimates for both normal and potentially sensitive individuals under a variety of possible exposure scenarios. These relationships can be examined at various analytical levels, each of which poses a series of scientific questions that must be answered before adequate quantitative models can be developed. The research efforts described below summarize the more extensive discussions and represent a research plan to address the major uncertainties surrounding methanol health effects. Currently there is no formal risk assessment or reference concentration assessment for methanol.

Exposure Assessment

- *Atmospheric exposure models must be developed and validated to enable estimation of the exposure concentrations along the distribution sequence to which humans might be subjected under various exposure scenarios.* Ambient and personal monitoring should be carried out both to validate the exposure models and to describe the distribution of exposures in specific microenvironments (with emphasis on the high-end exposures). The ideal end-product of these models or measurements would be a concentration-time profile detailing the exposure of the individual over an entire day.
- *Exposure dosimetry models should be developed to mimic anticipated exposure scenarios, making certain to include a broad range of projected microenvironments.*
- *The probabilities of other exposure routes, especially dermal, need examination.* A first step would entail extrapolation from current information about controlled and uncontrolled gasoline distribution and exposure.

Pharmacokinetics

Physiologically based pharmacokinetic models of methanol and formate disposition are probably the most useful quantitative models for translating exposure profiles, developed as described above, into dose at the target site. Validation of a model to predict formate accumulation as a function of target tissue dose is a critical requirement for predicting the impact of high and possibly moderate exposures. Incorporation into the model of both target tissues (the visual system, the nervous system, the testes, and the neural tube during early development) and target organ metabolism (activation and detoxification, if appropriate) would be essential for adequately predicting target tissue dosimetry. Such models should also be capable of describing the disposition of methanol and formate in potentially sensitive populations, such as those individuals with a folate deficiency. Predictions based on the relatively low ambient levels projected during most ordinary scenarios should not, however, be neglected, particularly for situations involving prolonged exposures.

- *Determine the pharmacokinetics of methanol and formate during pregnancy in the maternal/fetal unit in rats, mice, and nonhuman primates following single and repeated methanol exposures.* This work should be extended to animals with a reduced folate status to determine whether this affects methanol metabolism and to animals on a reduced folate diet to determine whether exposure to methanol alters folate metabolism and leads to a more rapid fall in folate levels.
- *Conduct human pharmacokinetic studies to develop a physiologically based pharmacokinetic model to help describe and quantify the disposition of methanol and formate in individuals who vary in folate levels at the anticipated exposure levels and durations.* Although experimentally induced folate deficiency is not a feasible strategy, correlating methanol and formate blood levels with individual folate levels after exposure to the methanol TLV of 200 ppm is feasible.
- *Determine target-organ-specific metabolism of methanol and effects (with particular attention to*

the brain, the testes, the retina, and optic nerve). Clarify the mechanisms of toxicity and susceptibility factors such as age and folate status.

- *Investigate formate elevation as a marker both of exposure and of effect in human populations, emphasizing occupational settings.*

Health Risks

Quantitative expressions linking dose, effects, and mechanisms are essential steps for estimating the risks of visual, developmental, or neurobehavioral toxicity resulting from exposure to methanol. These expressions will be most useful if they are based on dose to target tissue. In this way, the dose-response model can be linked with the emissions-exposure and exposure-dose models to estimate risk from relevant exposure scenarios.

In most scenarios, methanol exposures are not projected to attain levels high enough to produce marked elevations of formate in blood. The absence of high formate concentrations does not imply the absence of toxicity. Neurotoxicity is a special concern because currently available data suggest both changes in performance and adverse subjective effects, such as mood changes and irritation at exposures near the current TLV. These data are sparse and require amplification. Because the developing fetus may be at greater risk of methanol exposure, research is necessary to develop dose-response relationships for pregnancy outcomes in various species. In the case of the fetus, methanol exposure is also of concern because it may contribute to folate deficiency, which has been associated with a higher risk of spina bifida. This possibility needs to be investigated.

The visual system and visual function are altered as a consequence of methanol intoxication. Quantitative relationships between formate blood and target tissue levels and the onset and development of visual toxicity have not been established and should be characterized in any comprehensive risk assessment. The metabolic and nutritional factors that may contribute to the recognized variation in susceptibility to methanol intoxication have not been extensively investigated, nor have other factors, such as genetic variations or the effect of age. In particular, the possibility that protracted, low-level methanol exposure could cause effects similar to those of higher level

transient exposure requires exploration. In most cases, by using the appropriate animal models (i.e., animals with no elevated formate levels, animals with minor elevations in formate levels, and animals with severe elevations in formate levels), it should be possible to investigate the effects of both methanol and formate in any individual study.

- *Studies of developmental effects should be conducted, including neurobehavioral endpoints, in rodents and nonhuman primates with both normal and folate-deficient status. Folate deficiency levels should include values found in population surveys.*
- *Experimental human studies are necessary to provide a more comprehensive assessment of adverse neurobehavioral and visual consequences during and after short-term exposures. These studies should include a wider range of concentrations than the pioneering study by Cook and associates (1991) and more specific and sensitive measures of visual function, fine motor function, and memory. For example, the situation known as delayed matching-to-sample is probably a more efficient assay of memory than components drawn from typical neuropsychological tests.*
- *Chronic animal experiments, embodying neurotoxic, visual, and neuroendocrine effects are missing from the data base. Such studies should include normal animals, animals classified as folate-reduced, and animals classified as folate-deficient. One aim of folate reduction is to obtain an animal model that mimics the human situation in terms of recurring or chronic mild elevations in blood and tissue formate levels.*
- *Very few epidemiologic studies have been conducted; existing studies are too limited (e.g., no or poor exposure assessment) for interpretation. Worker populations that are currently exposed should be monitored for effects, particularly those related to actions on the central nervous system and on fetal development. Epidemiologic studies should also be aimed at the enduring consequences of poisoning episodes. The currently available information is based on gross clinical criteria. It should be expanded to include assessment of neurobehavioral changes.*

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Research Priorities to Reduce Uncertainties in Risk Assessment for Polycyclic Organic Matter

INTRODUCTION

Perhaps the most difficult aspect of dealing with polycyclic organic matter (POM) is the large number and diverse physical-chemical nature of compounds within this group. POM is defined in Section 112, Title II of the Federal Clean Air Act as a class of organic compounds having more than one benzene ring and a boiling point of 100°C or higher. This class includes polycyclic aromatic hydrocarbons (PAHs), substituted PAHs such as nitro-PAHs and alkyl-PAHs, heterocyclics such as aza-arenes and thio-arenes, and other subclasses such as lactones. POM compounds with five or more rings are generally associated with particles; those having four or fewer rings are semivolatile and are partitioned between the particle phase and gas phase. Consideration of POM must also include products of chemical and photochemical reactions in the environment. At present, the full spectrum of these transformation products is unknown; nor is it known what portion they constitute of the total airborne POM load. Because many POM compounds are thought to act through biotransformation into reactive metabolites, the complexity of the issue is increased by the vast number of potential metabolites that are formed after deposition in the body.

There are several sources for both the formation of these components and their release into the human environment, and only a portion of the total intake of POM is achieved by inhalation. POM compounds are ubiquitous products of incomplete combustion of organic matter and are encountered daily by all humans by ingestion in the normal diet and by inhalation of combustion products (e.g., tobacco, wood, and fossil fuels). Although mobile emission sources contribute a portion of the total human uptake of POM, there

may be few, if any, compounds unique to mobile sources. The portion contributed by mobile sources is certain to be variable and, at present, is poorly documented.

On the basis of current evidence as reviewed by the International Agency for Research on Cancer (IARC) (1984a, b, c, 1985, 1987, 1989), cancer is the potential health risk of concern regarding exposures to airborne POM. Many POM compounds are mutagenic; however, there is no strong evidence of heritable mutagenic or reproductive effects. Although the possibility of noncarcinogenic effects of POM cannot be excluded, there is no evidence to support further research in this area other than observations of potential noncancer effects in studies of carcinogenicity in animals or humans.

The potential magnitude of health risks from environmental exposures to POM is uncertain at this time. There is strong evidence that certain high-level occupational exposures to inhaled POM have caused increased incidences of lung cancer (IARC 1984a, b, c, 1985, 1987, 1989). Environmental exposures to airborne POM are ubiquitous, but exposure levels are low in comparison with the occupational exposures known to be carcinogenic, and confounding exposures, such as by ingestion, are also ubiquitous. Thus, although it is plausible that environmental exposures to airborne POM contribute to cancer among the general population, the existence or magnitude of such an effect may not be resolved by classical epidemiology. International working groups have concluded that environmental exposures to POM probably contribute to carcinogenesis in humans (Higginson and Jensen 1977; Holmberg and Ahlberg 1983; Pershagen 1990).

The following discussion of research needs is targeted toward resolving current uncertainties about the cancer risk of environmental exposures to POM from mobile sources. The U.S. Environmental Protection Agency (EPA) estimate of the contribution of particle emissions from mobile sources to the annual cancer incidence in the United States ranges from a few tens of cases per year to a few hundred cases per year (U.S. Environmental Protection Agency 1993). The largest portion of this mobile source contribution to cancers has been estimated to be due to particle emissions from heavy-duty diesel-powered vehicles. Excess cancer incidences of this magnitude would

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be extremely difficult to detect by epidemiology. Most of the uncertainties identified and the research approaches recommended to resolve the uncertainties apply to other sources of airborne POM equally as well as to mobile sources. The discussion is organized into sections on characterization of airborne POM and contributions of mobile sources, the toxicokinetics and health effects of POM, biomarkers of exposure and adverse health effects, and risk assessment. Research needs are summarized and priorities are identified at the end of the chapter.

EXPOSURE ASSESSMENT

Contribution of Mobile Source Emissions to POM in the Environment

A better approach is needed for characterizing biologically important changes in emissions from mobile sources as technologies change. Mobile source technologies (engines, fuels, emissions controls, etc.) continue to evolve. Much of the laboratory data supporting current cancer risk estimates were developed using engines and emissions controls that are no longer current. Technological differences can result in differences in emissions, which in turn can cause differences in the biological activity of emissions components (Lewtas 1983; Schuetzle 1983). The importance of these differences to human carcinogenic risk is not known. Studies correlating biological activity with chemical composition have indicated that emissions higher in PAH and nitro-PAH are higher in mutagenic and carcinogenic activity (Lewtas 1988). On the other hand, exposures of rats in different laboratories to whole diesel exhaust generated using different engines, fuels, engine operating conditions, and exposure patterns resulted in lung tumor incidences that were related directly to exhaust exposure rate. All studies had a similar relationship between exposure and response (Mauderly 1992). While it might be expected that overall cancer risk will be reduced as emission rates are reduced, the impacts of changes in the proportion of emission constituents are less certain. It is not practical to conduct a full range of biological assays, including long-term animal studies, to examine the effects of each technological change; thus, alternate strategies must be developed.

Research Recommendations

- *Research is needed to develop and validate approaches for determining when changes in technology produce sufficiently different emissions from those characterized previously to preclude predicting biological effects.*
- *A better quantitation of airborne POM by class of compounds, and a better understanding of the contributions of mobile sources to each POM class are needed.* Some studies of the composition of complex mixtures of POM have quantified the relative abundances of individual POM compounds and have divided them into groups based on the number of rings associated with their structure. Studies of the genotoxic potential of POM in petroleum distillates have suggested a correlation between carcinogenic potency and levels of POM compounds having three to seven rings (Blackburn et al. 1984; Roy et al. 1988a, b). Similarly, studies of the carcinogenic potential of several fractions of gasoline engine exhaust indicated that the fractions containing POM compounds having more than three rings were largely responsible for carcinogenic activity (Grimmer et al. 1983a, b). These findings suggest that POM mixtures having higher percentages of components with three to seven rings are likely to exhibit greater carcinogenic activity. An assessment is needed of the relative distribution of POM components containing fewer than two rings, three to seven rings, and eight or more rings in emissions from different types of light- and heavy-duty engines of current technology. These data would provide insight into the contribution of each source to POM components containing three to seven rings, as well as other classes.
- *It would be useful to determine the characteristic contributions of other mobile and stationary sources to different classes of POMs, in addition to understanding the contributions of automotive engines.* Emissions from off-road vehicles and equipment, locomotives, marine engines, and aircraft engines also contribute to airborne POM, and in some areas may be the predominant contributors. Certainly, stationary sources, such as industrial processes, the heating of buildings, and power generation, are frequent, and sometimes predominant, contributors. Only through some form of apportionment among these sources can the risk from light-duty and heavy-duty automotive

- engines and the benefits from reducing their emissions be adequately judged.
- *Improved analytical techniques are needed for evaluation of emissions from modern engines.* It is becoming increasingly difficult, using established methods, to obtain detailed measurements of the particle and POM emission rates of light-duty engines as the particle emission rates are reduced. It currently requires up to six hours of collection to obtain useful amounts of particles from some gasoline engines. Because the yield of particles is so low, the resulting amounts of POM are often at the lower limit of deductibility by established high performance liquid chromatography (HPLC)/gas chromatography-mass spectrometry (GC-MS) methods. It may become less important from a health perspective to fully characterize emissions if they are sufficiently low. If emissions are characterized, improved analytical methods that permit more sensitive detection and characterization of both particles and POM are needed. The particle emission rates from heavy-duty diesel engines are sufficiently high that the required characterization is still feasible with established methods.
 - *Detailed analytical studies are needed to provide quantitative data for levels in engine exhaust of some of the more potent carcinogenic POM compounds that have been identified as air contaminants.* These studies could provide information which may be useful for future investigations of surrogates, or representatives, of POM classes, as well as pointing toward compounds useful as biomarkers. This work would include quantitation of known carcinogenic agents such as benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene, dibenzo[*a,h*]anthracene, benzo[*j*]fluoranthene, cyclopenta[*c,d*]pyrene, and several isomers of dibenzopyrene. Recent studies have indicated that some dibenzopyrenes may have carcinogenic potencies which could be two orders of magnitude greater than BaP (Cavalieri et al. 1989, 1991; RamaKrishna et al. 1992). Studies providing quantitative data on dibenzopyrenes such as dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, and dibenzo[*a,l*]pyrene in both ambient air and engine emissions are critical for the assessment of these agents as mobile air toxics. Further quantitative data on the current levels of some of the more abundant POM compounds in ambient air and engine emissions are also needed, although emissions rates of POM from some current engines are at the lower limit of deductibility by present methods. These data are essential for understanding source apportionment of mobile source POM as well as atmospheric transformation and for exploring biomarkers of exposure.
 - *It is important to develop linkages between emissions from mobile sources and airborne POMs.* Airborne POM compounds are emitted from multiple sources, and more information on source apportionment under varying conditions is needed. An understanding of the relative contributions of mobile sources to airborne POM is key to determining benefits to be gained from controlling their contribution and to determining the most important chemical species to control. An example of one type of such studies is the apportionment of airborne mutagenic activity between mobile source and woodsmoke emissions (Lewtas et al. 1992, Cupitt et al. 1993). Other studies of this type are needed to examine sources of POM in different environments, with the goal of determining the extent to which generalizations are possible. This information will also help in the identification of representative POM components, or surrogates for them that are useful as markers, and it will help focus studies of toxicologically important atmospheric transformations of POM.

Contributions of Atmospheric Transformations

A better understanding of the atmospheric transformations of POM from mobile sources is needed. The body of information is growing, but the atmospheric fate of POM remains only partly understood. Our current understanding, however, suggests that chemical and photochemical reactions may result in chemical species of toxicologic importance. Research in this area has largely focused on a series of PAHs and their nitration and oxidation products (Atkinson and Arey 1993). It is important to understand the potentially toxic transformation products of some of the more abundant types of POM, in addition to those of PAHs present in small amounts. Although they are

supported to a limited extent, such studies need to be expanded and linked more firmly to human exposure and cancer risk assessments.

Research Recommendations.

- *Research on the transformations of both vapor phase and particle phase POM, and on interactions between the two phases is needed.* The 1988 HEI book entitled "Air Pollution, the Automobile, and Public Health" (Watson et al. 1988) reviewed the status of our understanding of the atmospheric transformations of automotive emissions including evidence that the atmospheric transformations of PAHs were dependent on the phase with which they were associated. The authors concluded that gas phase PAHs react with hydroxyl radicals and that this route may be important for the formation of nitro-PAHs, and therefore concluded that "a comprehensive and systematic investigation of the gas phase and particle adsorbed phase reactions of this class of automotive emissions is necessary before further risk assessment studies concerning these compounds can be carried out." The authors acknowledged the difficulty of this research endeavor, due to the partitioning of these emissions and their products between the gas and particulate phases. More recently, Atkinson and Arey (1993) reviewed advances in research on atmospheric transformation products. Although more information is needed on transformations, including those that might occur during sampling, we must also understand the significance of phase distribution for dosimetry and cancer risk.
- *Studies are particularly needed to characterize the more polar transformation products resulting from reactions of hydroxyl and nitrate radicals, photooxidation, and nitration.* Atmospheric transformations of primary diesel emissions produce additional nitro-PAHs that are mutagenic and may have potential health impacts. For example, Arey and associates (1992) have demonstrated the formation of nitropolycyclic lactones that are highly mutagenic. The polar PAH transformation products may represent a new and important class of mutagens. Arey, Atkinson, and their colleagues (Arey et al. 1986, 1987; Zielinska et al. 1989; Atkinson et al. 1988) have demonstrated that ambient air contains nitro-PAHs different from those found in fresh diesel exhaust.
- In a report to the California Air Resources Board, Winer and Atkinson (1987) documented ambient levels of 1- and 2-nitronaphthalenes and 3-nitrobiphenyl as high as 3.0, 2.9, and 6.0 ng/m³ in Torrance, California. These are thought to be products of atmospheric transformation. Atkinson and associates (1988) summarized average concentrations of ambient PAHs and PAH derivatives measured at selected sites in California. They reported that the particle-bound nitroarene in greatest concentration was 2-nitrofluoranthene, which is a product of the atmospheric transformation of fluoranthene. Atkinson and Arey (1993) listed the nitroarene products formed from gas phase reactions of PAH with hydroxyl and nitrate radicals, along with their yields. The lifetimes of these nitroarenes are greater than those of the parent PAHs, which may have important potential health consequences. This finding serves to point out the importance of understanding the lifetimes of transformation products in the atmosphere, as well as their concentrations.
- *A better understanding is needed of the partitioning between the gas and particle phases of atmospheric reaction products.* It appears likely that the products of gas phase atmospheric chemistry condense onto particles in the atmosphere (Atkinson and Arey 1993). To thoroughly understand measurements of human exposures to reaction products, it will be necessary to know whether the products are in the particle or vapor phase.
- *The biological significance of POM and derivatives of POM, especially the nitroarene compounds, produced by atmospheric transformations needs to be studied.* In addition to the identification, characterization of lifetime, and measurement of ambient concentrations for POM, the following studies are appropriate: in vitro mutagenicity (e.g., Ames assay), in vivo mutagenicity, chromosome alterations (e.g., micronucleus and sister chromatid exchange), carcinogenicity (skin and lung), and biomarkers (e.g., DNA and hemoglobin adducts). The priorities for bioassays should be driven by the data on ambient concentration, lifetime, and structure-activity relationships. The need for more

extensive health assays, such as carcinogenicity testing, should be driven by results of the *in vitro* and shorter-term *in vivo* assays.

The biological activities of nitropyrenes and several other nitro-PAHs (e.g., nitrofluorene and nitrochrysene) have been studied, leading to their classification by IARC as probably carcinogenic to humans. Little effort, however, has been directed toward assessing actual human exposure to these compounds and the resulting cancer risks. Little research has been directed toward the biological significance of the more abundant and volatile nitroarenes such as nitrobiphenyl (Nishioka and Lewtas 1992), which might be presumed to have biological activity similar to that of the known human carcinogen aminobiphenyl. Research is also needed on species having functional groups such as hydroxylated nitroarenes and nitrolactones, on isomers such as 2-nitrofluoranthene that are predominantly formed by transformation, and on isomers such as 3-nitrofluoranthene that are predominantly emitted directly.

- *Further research on the atmospheric chemistry of POM should be conducted in environmental chambers starting with known materials.* These investigations are helping identify reaction products that might be searched for in ambient air samples, and are also helping identify mechanisms of transformation. These data will facilitate the development of models that characterize the processes and could be used to predict reactions in ambient air. The materials produced by these studies could then be used in assays of biological activity. The results would facilitate bioassay-directed fractionation of ambient particulate extracts (Arey et al. 1992). The effort in this area needs to be expanded.

Human Exposures to Mobile Source POM

Research Recommendations.

- *Research is needed to develop an understanding of the total human exposure to POM by various routes of exposure.* Human exposure to POM has been estimated by measuring exposure to specific PAHs such as BaP or a sum of all PAHs. In only a few cases has the total solvent-extractable organic matter been measured in human exposure studies (Cupitt et al. 1993).

There are so few comprehensive studies of human exposure to POM that exposure to respirable particles has been used as a surrogate.

It is critical that an approach to measuring POM be developed that is compatible with the health effects data so that these may eventually be combined in a risk assessment. For example, if the health data will be based on organic material extracted using a dichloromethane solvent, then the exposure data should be collected measuring POM by the same extraction method.

- *Research is needed to apportion human exposures between various emission sources.*

A recent approach to this problem used a combination of receptor modeling and exposure modeling based on indoor and outdoor measurements and activity diaries in one city (Cupitt et al. 1993). Although this study apportioned exposures to extractable organic matter between wood combustion and mobile sources, the investigators were not able to apportion the exposure to different types of vehicles (e.g., diesel vs. gasoline). This type of information is needed on a regional and national scale. The development of improved mobile-source-specific tracer species is needed to attribute POM exposure to a specific mobile source category.

HEALTH EFFECTS

Toxicokinetics

Information is needed on the target tissue doses of POM compounds and their metabolites thought important for cancer risk. The metabolic pathways of BaP and some nitroarenes are fairly well understood, although uncertainties remain regarding toxicokinetics by different routes of exposure and about the role of non-bay region diol epoxide-DNA adducts. Furthermore, information on the toxicokinetics of other POM compounds is needed to determine their behavior by class. Because it will not be practical to construct toxicokinetic pathways and rate constants for each species, selected studies of representatives of classes of POM should be done. The grouping of POM compounds into toxicokinetic classes should parallel groupings used for bioassays and for predicting cancer risk.

Research Recommendations.

- *The behavior of particle-bound and vapor phase POM after deposition in the respiratory tract needs to be better understood.* Recent studies have provided new insights into the movement of lipophilic compounds deposited on airways and the influence of lipid surface layers on the spreading and clearance of PAHs (Gerde et al. 1993b, c, d). These experiments have given rise to conceptual models for predicting the behavior of PAHs on airway surfaces and their penetration into epithelial cells (Gerde et al. 1991, 1993a). More information is also needed on the extent to which POM is released from particles after deposition in the lung. Early work suggested that little of the organic fraction of soot was eluted in simple physiologic fluids such as saline, lung surfactant, or serum (Brooks et al. 1981, King et al. 1981). However, pulmonary macrophages are known to excrete metabolites of phagocytized BaP (Bond et al. 1984). It has also been shown that BaP (Sun et al. 1984) and 1-nitropyrene (Bond et al. 1986) are released from particles after deposition in the lung. The bioavailability of particle-associated POM after deposition in the lung was reviewed by Sun and associates (1988). Recent studies by Gallagher and associates (1992) and Randerath and associates (1992) have examined the usefulness of DNA adducts as a dosimeter for POM released from inhaled diesel soot. The extent of bioavailability of particle-associated POM remains uncertain, but is critical to understanding carcinogenic risk.
- *The potential role of ingested POM versus inhaled POM in lung cancer needs to be examined.* We do not understand the contribution of ingested POM to lung cancer induction, but it is plausible to postulate that it plays a role. Inhalation is not ordinarily the route of the greatest total intake of POM; however, inhaled POM is likely to contribute a greater lung cancer risk than ingested POM. POM is present in many baked, fried, and grilled foods, presumably as products of incomplete combustion, and the greatest intake is probably dietary. Moreover, it has been estimated that the intake of POM by ingestion may exceed the intake by inhalation even for particle-associated POM originally emitted as airborne emissions from mobile sources

(Cuddihy et al. 1980, Waldman et al. 1991). It must also be remembered that a portion of inhaled, particle-associated POM is rapidly cleared from airways and ingested. Finally, it must be considered that the ingestion of particles during grooming results in a substantial internalized dose of POM in any study of rodents receiving whole-body exposures to particle-associated POM (Wolff et al. 1982). Limited studies of POM applied to skin (Gallagher et al. 1990) or in the diet (Culp et al. 1992) suggest that the lung is a major site of DNA adduct formation after these exposures. More data are needed to determine the biologically effective dose to the lung from oral versus inhalation exposures to POM.

- *Information on the toxicokinetics of ingested POM and movement of POM compounds or their metabolites to lung is needed.* More information is needed on the conditions under which ingested POM could contribute to lung cancer. There are data demonstrating alterations of lung DNA in rats fed large doses of POM, but it is not known if ingested POM in dose ranges relevant to either animal inhalation studies or human exposures has any impact on the lung. Presumably, such an impact would act through the metabolism of POM compounds in the gastrointestinal tract or liver and the subsequent transport of metabolites to the lung. Ingested POM compounds undergo a first pass through the liver where they may be bioactivated and then reach other organs, where they may potentially present a carcinogenic risk. In contrast, the POM may be biotransformed in the liver, conjugated, and excreted. A third alternative concerns the metabolism of nitroarenes, which have been shown to be bioactivated in the gastrointestinal tract itself as a result of nitroreductase activity. Thus, the chemical dynamics of ingested POM are complex and may have significance with respect to the potential carcinogenicity of POM as well to the site specificity of the carcinogenicity.

Importance of Particle Association of POM

Incomplete combustion of engine fuels not only produces POM, but also produces very fine primary carbon particles (10-30 nm) that aggregate to form the soot measured in engine emissions. The amount of carbon particles produced varies

inversely with the efficiency of combustion and depends mostly on the ratio of air to fuel that the engine is burning at any given time. Because unburned fuel and engine oil are also entrained in the exhaust gas, the particle-bound POM does not originate solely from the combustion process. Furthermore, POM is only a small percentage of the total organic mass attached to the carbon particle; the largest portion typically consists of aliphatic compounds. The fact that POM in the ambient air is mostly adsorbed on the surface of small carrier particles raises the following questions.

Research Recommendations.

- *We need to know if the carcinogenic potency of POM chemically extracted from particles is similar to the potency of mobile-source-specific POM that reaches the lung adsorbed to the surface of small particles.* The long residence time of carbon particles in the lung (perhaps longer in humans than in rats) and the long-term release of POM from the particle may combine to prolong the exposure of target cells to POM and in this way produce a stronger carcinogenic effect. Because the amount of solvent-extractable organic material on the exhaust particles can account for up to 70% of the total particle mass (although more typically 5%-20%), the layer of POM on the particle is certainly many molecules thick. It is likely that the layers of POM in closest contact with the particle surface may be much more strongly bound to the particle than the outer layers. Little is known of the location of POM within the organic fraction adsorbed to soot, and whether it is distributed uniformly in the organic matter or preferentially in inner or outer layers. Thus, the bioavailability and biological half-time of soot-associated POM are poorly understood.
- *The possible toxic and carcinogenic effects of the carrier particles themselves in humans need to be better understood.* Inhalation studies with carbon black and titanium dioxide that, like diesel soot, consisted of agglomerates of primary particles smaller than 30 nm have shown carcinogenic effects in the rat lung (Heinrich 1993; Heinrich et al. 1993; Pott et al. 1993). Whether this effect is specific to rats exposed to produce a high particle load in the lung, which therefore may have no relevance

for humans, is still an open question (Mauderly 1990, 1993; Nikula et al. 1993). The carcinogenic effect of small nonfiber particles may well have a relevance also for humans, but the carcinogenic risk may be below the epidemiologic detection level. As long as it is not known by what mechanism the almost nonsoluble submicron-size particles cause lung tumors in the rat lung, and as long as it is not proved that this is a rat-specific mechanism, we cannot exclude that similar particle-related effects may occur in humans. Inorganic fiber particles, such as asbestos and some man-made mineral fibers, are also able to induce lung tumors in the rat. Because asbestos has been proved to be a human carcinogen, asbestos-related lung tumors in the rat are not usually regarded as a species-specific effect or as an effect due to overloading of the lung with fibers.

- *We need to know if the particle-specific and POM-specific carcinogenic effects of inhaled materials are interactive.* Assuming that both particles and POM are carcinogenic when inhaled, the question arises whether these effects are simply additive or more than additive. Inhalation experiments in rats with carbon black particles and coal tar-pitch condensation aerosol showed a synergistic carcinogenic effect of POM and carbon particles (Heinrich 1989; Heinrich et al. 1993). The question is whether similar effects may occur with carbon particles and mobile-source-specific POM.

Carcinogenic Risks from POM Mixtures

Cancer unit risk estimates have been developed for POM based on epidemiologic data for coke oven emissions (U.S. Environmental Protection Agency 1984), and for roofing coal tar emissions and cigarette smoke (Albert et al. 1983). These data show over two orders of magnitude difference in the cancer risks per microgram of solvent-extracted organic material. Several rodent cancer models have been used to evaluate the cancer potency of POM mixtures, including mouse skin (Nesnow et al. 1983), lung implantation (Grimmer 1985) and inhalation (Heinrich 1989; Heinrich et al. 1986b, c, 1989a, 1993). All of these cancer models show substantial differences in tumor potency of different POM sources (e.g., coal tars compared with diesel exhaust). Although the lung

implantation model has been used to evaluate the carcinogenicity of extracted organic material from both gasoline (leaded) and diesel exhaust, only the skin tumor initiation model has been used to compare different vehicles within one mobile source category (e.g., diesel exhaust).

The current data on the cancer potency of POM from mobile sources (diesel and gasoline exhaust) suggest they are at least one order of magnitude less potent as rodent lung carcinogens and skin tumor initiators than coal-tar-derived POM (e.g., from coke ovens or coal furnaces). Skin tumor initiation studies show that the POM compounds from different diesel vehicles exhibited different tumor initiation potencies (Nesnow et al. 1983). Inhalation studies with different diesel vehicles in different laboratories (Mauderly 1992) suggest that the cancer response in the rat lung is similar regardless of the vehicle and that the response may be related to the particle load in the lung.

Research Recommendations.

- *Research is needed to determine the cancer risk from the POM component of mobile source emissions as a function of different source factors (e.g., engines and fuels).* It may be possible that one cancer unit risk for POM, within a range, may adequately describe the cancer risk of all mobile source emissions, but it may also be that there are substantial differences among diesel vehicles versus gasoline, heavy-duty versus light-duty, and so forth. We need to be able to determine whether the emissions from a new technology are substantially similar to emissions for which the cancer risk has been previously evaluated. This research need is related to the need to evaluate the different animal cancer models available, because the different models (e.g., inhalation, lung implantation, and skin) have different sensitivities, advantages, and limitations.
 - *Research is needed to determine whether the use of single compounds (e.g., BaP), a group of compounds (e.g., sum of PAHs), or class fraction (e.g., aromatic fraction) could be used to calibrate and estimate the carcinogenicity of POM from mobile sources.* Studies by Grimmer (1985) suggest that the aromatic fraction of POM containing PAHs accounts for most of the carcinogenicity in both the skin and lung
- implantation models (except for diesel emissions, in which a nitro-PAH fraction accounts for a small fraction). Because some vehicles emit more unburned fuels and aliphatic noncarcinogenic compounds (e.g., some heavy-duty diesels) than other vehicles, it may be possible to measure the cancer response both in terms of total extractable material and in terms of BaP (PAH or aromatic fraction) content to determine if the variation of cancer risk of mobile source emissions is reduced (normalized) by expressing the risk per exposure to the sentinel chemical or chemicals. Such research would help to address the question of the role of the noncarcinogenic and non-POM components (e.g., aliphatic hydrocarbons) on the cancer risk of the whole emissions.
- *Research is needed to determine whether the POM not associated with particles (e.g., semivolatile two- to four-ring aromatics, nitro-aromatics, nitro-lactones) are carcinogenic in rodent models, and if so, what other contribution they make to the cancer risk of POM associated with the particles.* In some cases, the total mass emission factors and mutagenic emission factors of the semivolatile organics may be greater than the particle emissions. There are only limited animal cancer studies suggesting that the gaseous and semivolatile organics are not carcinogenic in rodent models (Heinrich et al. 1982, 1986a, 1989b; Brightwell et al. 1989).
 - *Research is needed to determine whether atmospheric transformation of mobile source POM emissions substantially alters the cancer risk from these emissions.* A number of studies demonstrate that nonmutagenic gaseous hydrocarbons and mobile source emissions are chemically transformed in smog chambers to produce mutagenic emissions, including some emissions that are not polycyclic in nature (e.g., peroxyacetyl nitrates) and others that would be classified as POM (e.g., nitro-biphenyl, nitro-aromatic lactones). Selected biological assays and perhaps some inhalation studies are needed to determine the carcinogenic potentials of these classes of compounds.
 - *A better understanding is needed of the synergistic and antagonistic relationships among POM compounds in causing health effects.* The difficulty in predicting the health effects of POM is illustrative of the broader problem of

understanding the effects of all chemical mixtures. Interactions among POM components in mixtures, and between POM components and other airborne materials, could be additive, synergistic, or antagonistic, and it is likely that all three relationships occur. There have been several studies of the effects of mixtures, although information on interactive relationships is sparse (Mauderly 1993). If representative POM compounds are to be selected for characterizing or regulating mixtures of POM, information will be needed on effects of mixtures and on their contributions to those effects, as well as on effects of the representative compounds alone. The effects of combinations of POM compounds should be examined in biological assays, beginning with the simpler assays and perhaps including selected inhalation carcinogenesis studies.

Utility of Biological Assays for Predicting Cancer Risk in Humans

There are several rodent models for evaluating the carcinogenicity of chemicals. The most widely used in evaluating both individual POM compounds and complex mixtures of POM are the mouse skin initiation and complete carcinogenesis models (Boutwell 1964) and the lung implantation model (Deutsch-Wenzel et al. 1983). Nitro-PAHs, which are relatively inactive on mouse skin, have been shown to be carcinogenic by lung implantation (Tokiwa et al. 1990) and in the newborn mouse model (Wislocki et al. 1986). Inhalation data are limited to one study of BaP in Syrian golden hamsters (Thyssen et al. 1981), coal tar pitch in rats (Heinrich et al. 1986b, c, 1991, 1993), diesel exhaust in several species (Mauderly 1992), and a variety of studies of cigarette smoke. A literature survey comparing the tumor responses of 3 species to BaP and 3-methylcholanthrene administered through different routes of exposure found respiratory tract implantation or infusion and skin administration to be more sensitive to tumor formation (Muller, 1991).

Research Recommendations.

- *Further comparisons of carcinogenic risks extrapolated from different animal cancer models to known risks among exposed humans are*

needed. Lifetime rodent inhalation studies are generally considered the best cancer model for human inhalation exposure of air pollutants. The high costs and time to conduct such studies will most likely preclude the evaluation of a large number of POM compounds from mobile source emissions by lifetime inhalation studies. Because epidemiologic data are available for a small number of POM sources (coke oven and cigarette smoke) for which animal data are also available in at least three models (mouse skin, rat lung implantation, and rat inhalation), it should be possible to evaluate these three models against the human data by independently estimating the human cancer risk from each data set.

- *Research is needed to evaluate the dosimetry, target cells, and mechanism of action of POM in each of the animal cancer models in comparison to our understanding of the mechanism of lung cancer induction in humans by POM.* This information is needed to gain a better understanding of how the dose-response functions and mechanisms of carcinogenesis in experimental systems can be used to improve risk assessment of POM through biologically based dose-response modeling (Moolgavkar and Knudson 1981, Thorslund et al. 1987, IARC 1992). This research need is broad because it encompasses the need to better understand the mechanisms of POM-induced carcinogenesis in humans, which requires an extension of our understanding of the fundamental processes of carcinogenesis. It includes the role of DNA adducts as dosimeters and markers of DNA injury, the relationship between mutagenesis and carcinogenesis, the role of POM in nonmutagenic cancer mechanisms, and the molecular basis for POM-induced carcinogenesis.

Linkages between mutagenicity and carcinogenicity need to be better understood. Because PAHs and nitro-PAHs that are carcinogenic are also typically mutagenic in *Salmonella*, the *Salmonella* mutagenicity assay has been used to identify potentially carcinogenic substances in incomplete combustion products. During the last several years, mechanistic studies have lent support to this approach. The process of chemical carcinogenesis has been shown to be a multistage one that may include the mutagenic

activation of oncogenes or the inactivation of suppressor genes. Because of this, and because the majority of known human carcinogens are mutagens, mutagenicity appears to be an important attribute of potentially carcinogenic POM.

Recent studies have suggested that the induction of mutations in bacteria by PAHs may occur by a different mechanism from that for the induction of cancer in mammalian cells: that is, metabolism to K-region versus bay-region epoxides. It is suggested, therefore, that in view of the importance of mutations both for the identification of carcinogens as mutagens and for the mechanism of carcinogenesis, a comparison should be undertaken to relate the mutagenic spectrum (base specificity; species affected; genetic mechanism involved such as point mutations, chromosomal aberrations, etc.), and the mutagenic potency to the carcinogenic spectrum and potency of POM. Much of the experimental data for such a study have already been generated. Such a study may lead to a better definition of carcinogenic POM as well as to possible predictive capabilities based on structural concepts.

Not all carcinogens are mutagens; thus, the possibility that POM compounds might induce cancer through nonmutagenic mechanisms must be considered. There is no strict correlation between mutagenic and carcinogenic potencies except within very narrow chemical classes. Cell proliferation (mitogenesis) has been identified as a potentially important contributor to carcinogenesis (Ames and Gold 1990). Assays for mitogens are being developed. Given the wide distribution of POM in emissions from mobile sources, there is a need to determine the mitogenic activity of nonmutagenic fractions present in such mixtures. This, in turn, may lead to the identification of new biologically active POM compounds.

Underlying all of the above information needs is the need to understand the basis for POM-induced carcinogenesis. An improved understanding of the relationship between the action of POM in nonhuman bioassays and potential human effects will probably be based on advances in our understanding of the molecular mechanisms by which the effects occur. Although this issue is certainly not specific to POM, it is important that consideration of POM be given in research on the mechanisms of carcinogenesis and other effects. Certain PAHs have been shown to activate *ras* oncogenes by mutation (Marshall et al.

1984, Dandekar et al. 1986). However, few consistent patterns have been demonstrated between toxic agent and genetic changes; indeed, the species of the subject and the tissue examined often appear to be as important influences as the species of toxic agent (Zarbl et al. 1985). Some examples of carcinogen specificity in oncogene activation have been found (Garte et al. 1985, Quintanilla et al. 1986), however, and work on agent-specific responses continues. An example of a biological test system that might be useful for examining effects of individual POM compounds and mixtures is the strain A mouse, which could yield information on correlations among POM, specific adducts, and specific gene alterations. These correlations could then be examined in inhalation studies for selected POM.

BIOMARKERS OF EXPOSURE AND ADVERSE HEALTH EFFECTS

Research Recommendations.

- *Research is needed to evaluate the utility of biomarkers of exposure and effects in studies in which personal exposure is linked to the appearance and level of biomarkers.* Biomarkers of exposure and genotoxic effects of POM have been developed and applied to human studies of cigarette smokers and individuals exposed to high levels of industrial PAHs (coke ovens, etc.) These biomarkers range from measures of protein and DNA adducts to cytogenetic and mutagenic effects in blood cells. Only recently have biomarkers been used to demonstrate exposure and potential adverse effects from exposure to urban air pollution (Perera et al. 1991) in a highly polluted region where summer and winter differences were observed. This research needs to focus on the relationships among biomarker levels, disease outcome, and human risk.
- *We need to understand the relationship between external exposure and markers of dose and effect in the target organ.* Research is needed to develop an understanding of the relationships between external personal exposure and internal exposure in the target organ as measured by biomarkers (e.g., protein adducts), target dose (e.g., DNA adducts), and genotoxic effects (e.g., cytogenetic damage and

mutations). These relationships may be complex because the target organ of concern is the lung and POM exposures from engine emissions and other sources may occur from multiple sources (e.g., home heating, diet) and via multiple routes of exposure (e.g., oral and inhalation).

- *Research is also needed to improve the sensitivity of biomarker methods for application to realistic environmental exposures.*
- *Effort should be made to take advantage of the growing field of molecular epidemiology to assess the health impact of POM directly by examining molecular biomarkers in humans.* The field of molecular epidemiology seeks to employ biomarkers to assess the effects of exposure directly, using samples from humans, rather than estimating effects from results in other assays (Perera et al. 1991). The goal of this field is to develop objective, specific, quantitative, and sensitive methods based on biochemical or molecular analyses, to assess exposure, doses in target tissues, early effects, and prognosis. To the extent that such markers are validated, this approach circumvents the problems of interspecies and high-low dose extrapolations. Considerable work remains to develop reliable approaches; current assays of DNA adducts and mutagenic spectra are not sufficient by themselves for the purpose of risk assessment (Gonzales et al. 1991, Cosma et al. 1992). The issue of individual differences in expression of markers (Harris 1989) needs to be resolved through consideration of variations due to gender, age, race, diet, and smoking. Genetic markers such as restriction fragment length polymorphism in genes such as cytochrome P-450, glutathione transferase, and others may provide diagnostic tools to better understand the relationship of individual variation to individual susceptibility (Nakachi et al. 1991, Tefre et al. 1991, Cosma et al. 1993). Theoretical issues such as statistical relationships between biomarker assay results, disease endpoints, and confounders of response will require careful study.

RISK ASSESSMENT METHODOLOGIES

Research Recommendations.

- *Research is needed to evaluate the various approaches for risk assessment of POM and to provide recommendations for improvements in the risk assessment methodologies.* The quantitative risk assessments used for POM have included a wide range of methods such as statistical extrapolation from human epidemiologic data, extrapolation from animal inhalation data, a comparative potency approach using both human and animal data on POM mixtures, and a BaP "toxic equivalence factor" approach. These methods use different underlying hypotheses and different methodologies. Although several investigators have compared these methods, no concerted effort has been made to perform a rigorous evaluation and comparison of these different approaches for the assessment of the cancer risk from POM.

This effort should include evaluation of the use of "relative potency factors" for individual PAHs (e.g., toxic equivalence factors). Consideration should be given to applying biologically based dose-response modeling to the risk assessment of POM. This effort should also include the quantification of uncertainties associated with the cancer risk assessment of POM. Consensus needs to be developed in the scientific community as to the validity and utility of the various options for assessing the risk of POM for different purposes (e.g., risk prioritization, technology evaluation, and standard setting).

PRIORITIZATION OF RESEARCH RECOMMENDATIONS

The discussion above points out a number of information needs regarding the health risks of airborne POM from mobile sources. The following is a more succinct summary of key research needs, based on the foregoing information. This list is not given strictly according to priority; however, high-priority items are indicated as such, and those that can largely be met in the next three years are also noted as "near term." These appear at the top of each list.

Characterization of Exposures to POM and Contributions of Mobile Sources

- *Better (more sensitive) methods of measuring vehicle emissions. Near term.*
- *Comparative analyses of atmospheric samples and emissions on the basis of a few selected (macroscopic) classes of POM.*
- *Identification of representative compounds in classes with the highest potency \times prevalence product, to be used as surrogates or sentinel compounds.*
- *Development and validation of methods for apportioning sources of human exposure to POM between mobile sources and other inhalation and noninhalation sources.*

Health Risks from POM

- *Examination of the carcinogenicity (particularly in lung) of ingested particle-bound POM. High priority; near term.*
- *Conduct of cross-validations among different bioassays, in comparison with known human carcinogen benchmarks. High priority; near term.*
- *Development of a better understanding of the uptake and distribution of POM compounds other than BaP, focusing on carcinogens. Near-term.*
- *Study of the distribution of adducts among tissues for POM compounds shown to be carcinogens, to estimate potential effects in nonlung sites, including lung and nonlung adducts from inhaled POM. Near term.*
- *Clarification of the link between mutagenicity in bacteria and carcinogenicity in humans. Near-term.*
- *Comparison of the carcinogenicity of POM compounds from mobile sources, with the goal of determining the degree of difference or similarity of the carcinogenic potencies of different emission sources.*
- *Apportionment of carcinogenicity among chemical classes of POM and individual POM compounds.*
- *Evaluation of whether POM not associated with particles presents a cancer risk.*
- *Determination of whether or not atmospheric transformations alter the cancer risk from mobile source POM.*

- *Determination of the metabolism of any POM, or transformed POM, that is carcinogenic, and for which we do not already know the metabolism.*
- *Comparison of noninhalation assays to inhalation assays for POM on particles versus POM off particles.*
- *Determination of the bioavailability of particle-bound POM, including different particle types, different mass loading, and different POM compounds.*
- *Examination of the relative carcinogenicities of POM compounds on particles and particle-free POM (by classes or by specific compounds). Include noncarcinogenic POM compounds on particles to simulate correct particle characteristics.*
- *Development of confidence that mechanisms by which effects occur in bioassays can occur under relevant conditions in humans, if the bioassay data are to be used directly for risk assessments.*
- *Testing of nonmutagenic POM compounds in assays for effects not mediated by mutagenicity.*
- *Exploration of the dose-response relationships of any bioassay effect used to estimate cancer risk, in order to ensure that responses at relevant doses are known.*

Biomarkers of Exposure to POM and Adverse Health Effects

- *Assurance that consideration is given to POM in efforts to develop new molecular epidemiologic tools. High priority; near term.*
- *Exploration of the relationship of biomarkers in blood, urine, and so forth, to the dose in lung, including consideration of dietary POM. High priority; near term.*

Risk assessment

- *Systematic comparison of the risk factors generated using different approaches, with the goal of achieving consensus on the best approach. High priority; near term.*
- *Effort to assess the uncertainties in any risk assessment method to be used for estimating risks in humans and developing risk management strategies. High priority; near term.*

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Cross-Cutting Issues

INTRODUCTION

There are a number of issues common to the investigation of the health effects of the mobile source air toxics. For all the pollutants discussed in this report, the primary research goal should be to develop and quantify the relationship between exposure, dose, and effects. In this context, obtaining reliable exposure data is the first necessary step. The issue of dosimetry, that is the consideration of physiologic and biochemical factors that may affect the amount of the pollutant delivered to the target tissue, needs also to be addressed. This issue is often tied to that of extrapolation, not only from high exposures to low exposure, but also among species. Lastly the approaches most suitable to addressing the question of health effects need to be broadly discussed. In this chapter, molecular biology and epidemiologic approaches are highlighted as being potential useful research tools for providing better data for risk assessment. While cancer has often been the endpoint considered in the risk assessment of many of the air toxics as well as for many other environmental pollutants, there is a broad recognition that noncancer endpoints, such as neurotoxic, reproductive, and developmental effects should also be evaluated. In this chapter, the cross-cutting issues of exposure assessment, dosimetry and interspecies extrapolation, extrapolation from high to low doses, molecular biology, epidemiology, and neurotoxic, reproductive and developmental effects are discussed.

EXPOSURE ASSESSMENT

Research to determine the risk of adverse health effects associated with toxic air pollutants emitted by motor vehicles, through combustion or evaporation, or produced by atmospheric reactions, should determine the intensity of human exposure.

Cross-Cutting Issues Experts: Paul J. Liroy (exposure assessment), Henry d'A. Heck (dosimetry and interspecies extrapolation), Daniel Krewski (extrapolation from high to low

doses), Seymour J. Garte (molecular biology), Genevieve M. Matanoski (epidemiology), and Thomas M. Burbacher (neurotoxic, reproductive, and developmental effects).

If there are situations or events that lead to acute or chronic human contact with specific chemicals or mixtures, we should define the contributions that result in a meaningful or de minimis exposure:effect ratio for cancer and noncancer endpoints. To accomplish such a task, we should identify the microenvironments that people occupy and that contain pollutants. We must also identify the ways in which a pollutant enters the body and sum the total exposure from the ingestion, inhalation, and dermal routes. Finally, we must develop profiles of individuals' exposure and duration activity patterns within the selected microenvironments.

There can be many different sources of toxic air pollutants, and the incremental increases to total exposure need to be quantified for vehicular combustive and evaporative emissions. This will place the significance of vehicular source emissions and pollutant accumulation patterns in perspective when attempts are made to estimate the total risk associated with a chemical. Total exposure analyses should include estimates or measurements of mean or median exposure for the general population as well as the high-end events, above the 90th percentile, of an exposure distribution. The distribution of incremental motor vehicle exposure must be determined for each specific route of entry into the body to estimate the incremental potential body burden. For an individual fuel, analyses of a probabilistic exposure distribution can have similarities for each route, but we must recognize that the pollutant mixtures in the unburned fuel and in the combustion products may be quite different. For such cases, knowledge about the biological effects of the pollutant or class of pollutants must be coupled with the concentration, the time of contact, and the other metrics used to quantify human exposure.

To complete analyses for specific "new" or "alternative" fuels, we must initially rely on information associated with the potential hazards. These data can be used to identify the potential contribution from inhalation, as well as other routes of exposure, and the activities that lead to biologically relevant human contact with the contaminants. Such information will increase the likelihood that we can adequately estimate a potential distribution of inhalation and other

exposures. In the case of a material that has not been introduced to commerce, all possibilities for long-term and acute contact must be explored in initial modeling exercises.

For all fuels and emissions products, we need to couple exposure estimates and measurements with toxicokinetic modeling to ensure that realistic estimates of internal dose can eventually be used for the characterization of risk. Such combined analyses will significantly improve exposure-dose-response analyses and will assist in defining the markers of external exposure and internal dose that are required for long-term assessment of exposure in the general population and in susceptible subgroups.

Issues and Uncertainties

For the individual air toxics included in this document, there are specific needs identified and gaps illustrated in the research area of exposure assessment. As might be anticipated, the needs are not necessarily the same because some of these compounds are almost exclusively combustion products (1,3-butadiene, formaldehyde, polycyclic organic matter [POM]), others are part of the raw fuel (methanol), and benzene is found as both. To further complicate the issue, methanol is primarily in the developmental stage as an alternative fuel, and POM is a complex mixture that includes a variety of hazardous components.

From the above, it is apparent that the locations (microenvironments) and types of human contact (inhalation, dermal, ingestion) in which high exposures occur can be different. However, important considerations for all these materials are the quantification of meaningful environmental exposures and attempts to relate this information to the pharmacokinetic process. Coupling of these data can lead to estimation of a biologically effective dose and to reduced uncertainty in risk assessment. A large exposure data base is available for benzene, and important exposure information is available for individual components of POM. For the other compounds, data on exposure in the general population or subgroups of the population are very limited. However, the situation is not simple, because aldehydes, benzene, and POM have numerous sources, and the incremental exposure associated with fuels and fuel combustion products must be differentiated from the total exposure. The case of methanol is unique in that

the fuel is not currently in wide distribution. Thus, focused microenvironmental and personal monitoring studies should be conducted in situations where methanol fuels are currently being used on a limited basis (e.g., fleet automobiles).

Another point of consensus in this workshop report is the need to develop biological markers of exposure that relate the external exposure from one or more routes to the internal or biologically effective dose. These types of data can, at a minimum, be useful in physiologically based pharmacokinetic (PBPK) studies and, if not too invasive, can be considered for use in routine testing of high-exposure groups.

From a population-based view of exposure, the working groups' recommendations attested to the need for understanding general population exposure. The detailed studies that were suggested should provide the basis for the development of large-scale studies that define the mean and high-end exposure subgroups.

To conduct any of these studies better methods are needed to measure air concentrations and to sample the study population.

DOSIMETRY AND INTERSPECIES EXTRAPOLATION

Predictions of human risk based on toxicologic data in laboratory animals involve major extrapolations across species and exposure levels and frequently across target tissues and exposure routes. Because these extrapolations often fail to incorporate relevant mechanistic information such as species differences in metabolism or in toxic response, nonlinearities in delivered dose or in cytotoxicity, or because critical mechanistic data are lacking, the resulting risk estimates may be very inaccurate. To incorporate more mechanistic information into risk assessments, an understanding of target tissue dose in different species under a variety of exposure conditions is basic.

The requirement for accurate and reliable dosimetry data is a common theme expressed in all of the workshop groups that attempted to define research needs for improving risk assessments for air toxics derived from mobile sources. Dosimetry studies in experimental animals are critical for the understanding of several generic risk assessment

issues, in particular, species differences in response, extrapolations from high to low doses, acute versus chronic exposures, and the effects of single compounds versus mixtures. Dosimetry studies using biomarkers in humans, although less feasible than in animals and more limited with respect to the types of information that can be obtained, are recognized to be potentially very useful as an index of exposure and of individual susceptibility. The goal of this article is to present an overview of the major generic dosimetry issues behind the research recommendations for each of the five chemical groups discussed in this report.

Species Differences

The need for a better understanding of species differences in susceptibility is exemplified by benzene, which is a known leukemogen in humans but does not appear to cause acute myelogenous leukemia in any of the standard laboratory animal models, although it does cause aplastic anemia and certain forms of cancer such as Zymbal gland tumors in animals. The lack of an adequate animal model has presented a major barrier to attempts to study the mechanism of benzene-induced leukemia. The extent to which differences in species susceptibility can be accounted for by differences in pharmacokinetics (delivered dose or metabolism) or to innate biological differences is unknown and remains an important research issue.

Another example of species differences is provided by methanol, which can cause ocular toxicity in humans and nonhuman primates but has no known toxic effect on the visual system of rodents. The reason for the differences in this case is believed to be the accumulation of formate, the major metabolite produced from methanol, to a toxic concentration in primates. Rodents are better able to convert formate to carbon dioxide owing to their faster rate of formate clearance. Laboratory studies have shown that the folate-deficient rat, in contrast to the normal rat, is susceptible to methanol toxicity. Thus, the difference in susceptibility between rats and primates appears to be primarily an effect of formate dose.

Mice are known to be much more susceptible than rats to the carcinogenic effects of butadiene. Large differences in the rates of metabolism of butadiene to monoepoxides and diepoxides exist between mice, rats, monkeys, and humans. Human metabolism both *in vivo* and *in vitro* appears to

resemble the monkey's more than rodents'. The lack of quantitative data on target tissue dosimetry of butadiene and its epoxides is a major cause of uncertainties in the development of physiologically based pharmacokinetic models for butadiene in humans.

Species differences in dosimetry also play an important role in the toxicology of two aldehydes: formaldehyde and acetaldehyde. Whereas the toxic effects of formaldehyde are essentially limited to the nose in rodents, the toxic effects in the monkey respiratory tract are more widespread, occurring in regions as deep as the carina. A more complete understanding of the factors affecting aldehyde absorption in the respiratory tract is recognized as an important research need for decreasing the uncertainties of aldehyde risk assessments.

In the case of POM, there is a need to better understand species differences in carcinogenic response and to develop appropriate biomarkers for human and animal exposure. Sensitive markers of dose in target and nontarget (surrogate) tissues, such as blood proteins or lymphocytes, should be developed, and research should be focused on the relationships between biomarker levels, disease outcome, and human risk.

Extrapolations from High to Low Doses

Dosimetry data are essential for the development and validation of models that can be used to extrapolate from high to low doses. This issue is addressed in the next section and will not be considered in detail here. However, it is important to note that dosimetry data can often be obtained at concentrations well below those that produce measurable effects. Such data are needed to define the shape of the dose-response curve in the low-dose region. Information about DNA adducts in the case of butadiene or POM, or DNA-protein cross-links in the case of aldehydes, can have a major impact on estimates of human risk. For example, data obtained on DNA-protein cross-links obtained over a wide range of concentrations in rats and monkeys exposed to formaldehyde were utilized by the U.S. Environmental Protection Agency and California Environmental Protection Agency to derive risk estimates for formaldehyde in humans. Using these data, the U.S. Environmental Protection Agency (1991a) reduced its earlier upper-bound unit risk estimate for formaldehyde-induced cancer 50-fold.

Acute Versus Chronic Exposures

In most cases, dosimetry data have been obtained after an acute exposure to a toxicant. However, the normal pattern of human exposure is chronic rather than acute. Moreover, bioassays in rodents are usually conducted for periods as long as 2 years. For several of the chemicals of interest to this review, including the aldehydes, methanol, butadiene, and POM, there is a recognized need for more information about dose in relation to the time course of toxic responses. Dosimetry studies during chronic exposures are especially important for carcinogens, owing to the long time lag between the initiation of exposures and the development of toxic responses, and the complex preneoplastic changes that can occur in tissues during chronic exposures to toxicants. These changes may result in significant alterations in the concentrations of reactive intermediates or levels of DNA adducts, which may lead to changes in the estimates of dose and significant modifications of risk assessments.

Chemical Mixtures

One of the most important generic dosimetry issues remaining to be addressed is that of chemical mixtures. "Real-world" exposures are never to single chemicals, which have dominated toxicology studies to date. In only a few cases, such as POM, have attempts been made to systematically approach the measurement of dose in a setting that approximates actual environmental exposures.

The approach to studying POM is unique in many ways. The exact composition of such a complex chemical mixture is not clearly defined. The composition of POM can be discussed most effectively in terms of chemical classes, such as polycyclic aromatic hydrocarbons and nitroarenes. Approaches to the measurement of dose, such as the measurement of DNA adducts, require the use of relatively nonspecific techniques, such as ³²P-postlabeling or immunochemical assays. Dose-response relationships can become exceedingly complex, with the occurrence at some concentrations of dose-response curves with negative slopes. The role of particles in the delivery of POM to target sites also becomes an important issue.

Exposures to single chemicals, such as benzene, butadiene, and methanol, are recognized to be in

actuality exposures to mixtures, because the compounds are metabolized to a variety of products with differing toxicities, and because they are present in the environment in combination with other pollutants. It is well known that compounds that are metabolized by the same isoenzyme of cytochrome P-450 can inhibit the biotransformation of one another. Thus, phenol and hydroquinone, two metabolites of benzene, can inhibit the metabolism of benzene. Interactions among benzene metabolites, quinones and aldehydes, have been suggested to account in part for the myelotoxic effects of benzene. Yet to be investigated are the possible effects of benzene and butadiene, or methanol and formaldehyde, on the toxicology of each other.

The aldehydes, formaldehyde and acetaldehyde, are a class of compounds that may well exhibit important mixture effects. Both compounds can be detoxified by the same enzyme, aldehyde dehydrogenase, which is present in the respiratory epithelium, but acetaldehyde has a higher affinity for this enzyme than formaldehyde does. If aldehyde dehydrogenase is a critical enzyme in the detoxication of formaldehyde, a simultaneous exposure to both compounds may inhibit the metabolism of formaldehyde and increase its toxicity. Other compounds present in ambient air, such as ozone and acrolein, have already been shown to enhance the toxicity of formaldehyde or to increase the amount of formaldehyde bound to DNA. Thus, mixture effects could play an important role in the toxicology of the aldehydes and possibly of other air toxics.

Dosimetry Studies in Humans

The discussion thus far has focused primarily on dose measurements in experimental animals. Obvious limitations exist on our ability to apply such approaches to humans. However, measurements of dose in human surrogate tissues, that is, biomarkers, are now feasible for a number of the compounds discussed in this document with the possible exception being the aldehydes. A critical need exists for more information about the relationship between biomarkers and actual exposure concentrations.

Several of the workshops pointed to the need for animal data that can be incorporated into physiologically based pharmacokinetic (PBPK) models. Measurements of dose in experimental

animals interpreted in terms of PBPK models have in many cases yielded reasonably close and, sometimes, quantitative estimates of dose in humans or other species, when the estimates have been validated. Information is needed about physiologic parameters (breathing rates, blood flows, partition coefficients) and metabolic rates (maximal velocities and Michaelis-Menten constants). The possibility of estimating human metabolic rates *in vitro* by using isolated human hepatocytes or liver slices offers a particularly promising avenue to obtain detailed information on human metabolism that cannot be obtained *in vivo*. The resulting measurements can then be incorporated into PBPK models to estimate rates of human metabolism.

Conclusions

Dosimetry as a cross-cutting issue impacts all of the chemicals discussed in this report in similar ways. Dosimetry studies in experimental animals are clearly needed to understand species differences in response and to guide extrapolation from high to low doses. There is a major gap, however, in our understanding of the relationship of subchronic exposures to delivered dose and in our comprehension of the effects of chemical mixtures. Future studies should focus more attention on combinations of chemicals that more closely approximate "real-world" exposures. Such studies could have a significant impact on our understanding of toxicologic mechanisms and could bring about a major improvement in the accuracy of risk assessments.

EXTRAPOLATION FROM HIGH TO LOW DOSES

Toxicologic or epidemiologic investigations of the adverse health effects of toxic air pollutants generally provide information for relatively high levels of exposure. High doses are necessary in laboratory studies to induce experimentally measurable rates of response in small populations of laboratory animals. Similarly, to maximize the opportunity of observing adverse health effects of exposure to toxic chemicals, epidemiologic studies often focus on occupational groups subjected to higher exposures than the general population.

Because of the high exposures in both toxicologic and epidemiologic studies, predictions of risk at lower environmental exposures generally require extrapolation from high to low doses. With toxicologic data, prediction of risks to humans also entails extrapolation from nonhuman test systems. Extrapolation from high to low doses raises a number of generic issues common to all of the chemicals examined in this report.

Biologically Based Dose-Response Models for Carcinogenesis

The linearized multistage (LMS) model has traditionally been used to extrapolate carcinogen bioassay data from high to low doses. This model is based on the notion that a cancerous cell is formed from a single somatic cell after the completion of a series of genetic alterations. Exposure to a carcinogenic agent is generally assumed to increase the mutation rate associated with one or more stages in a linear fashion. A consequence of this latter assumption is that the dose-response curve for the multistage model will be linear in the low-dose region. Because the corresponding linear term in the model may not be apparent when fitting the model to experimental data, a linearized form of the model, based on an upper confidence limit on the slope of the dose-response curve at low doses, is used in practice.

The multistage model is sufficiently flexible to describe a wide range of dose-response relationships observed in toxicologic and epidemiologic studies of carcinogenesis, but it does not take into account all of the factors known to be important in neoplastic development. Specifically, cell proliferation is known to play an important role in the induction of many cancerous lesions but is not taken into account in the LMS model. Tissue growth and development is another important factor that is ignored in applications of the LMS model. Other factors that warrant consideration in cancer modeling are DNA repair, early biochemical changes associated with preneoplastic lesions, formation of reactive metabolites, and receptor-mediated events such as the induction of cytochrome P-450 in the liver by binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to the Ah receptor.

As knowledge of the mechanisms of chemical carcinogenesis accumulates, it seems natural to incorporate this knowledge in more biologically based dose-response (BBDR) models of neoplastic

transformation. For example, the two-stage clonal expansion model of carcinogenesis explicitly takes into account both tissue growth and cell kinetics as well as DNA reactivity, envisaged as two critical mutations required to transform a normal cell into a malignant cancer cell. Although more elaborate BBDR models may be required to describe the mechanism of action of specific carcinogens, the two-stage clonal expansion model does attempt to incorporate key variables involved in carcinogenesis (i.e., tissue growth, cell kinetics, and DNA damage) and has been successfully used to describe the carcinogenic effects of substances such as tobacco smoke, radon, and certain nitroso compounds (Krewski et al. 1992).

BBDR models provide a useful framework for the quantitative description of carcinogenesis, but additional data are required to determine all of the unknown model parameters. This is because tumor incidence data used to fit simpler models, such as the LMS, contain no direct information on critical factors such as cell proliferation. Direct information on cell proliferation has been obtained from studies of cell kinetics with formaldehyde and should be sought for other compounds for which a BBDR model is to be constructed. Similarly, studies of DNA reactivity in which DNA adducts or protein cross-links are measured may provide information on mutation rates in the two-stage model. Data on tissue growth and development as a function of age can also be directly incorporated within the two-stage model.

If the mechanism by which tumors are induced is sufficiently well understood, and direct information on all of the model components can be obtained, it may be possible to construct a complete BBDR model without making use of tumor incidence data from toxicologic or epidemiologic studies. The model could then be validated by comparing model predictions of tumor incidence with observations from toxicologic or epidemiologic studies.

In principle, the same dose-response model can be applied to both toxicologic and epidemiologic data. In practice, the lack of accurate exposure data may preclude formal dose-response modeling with certain compounds such as butadiene. BBDR models have not yet been widely applied to epidemiologic data. Simple linear or linear-quadratic models have been used to describe the relationship between occupational exposure to

benzene and the risk of leukemia among pliofilm workers.

Physiologically Based Pharmacokinetic Models

Many compounds require metabolic activation to exert their carcinogenic effects. Physiologically based pharmacokinetic models provide a biologically based framework for describing the pharmacokinetic disposition of the parent compound in the body and the formation of reactive metabolites. PBPK models envisage the body as comprising a small number of physiologic meaningful compartments and are characterized by physiologic, biochemical, and metabolic parameters that govern the delivery of reactive metabolites to target tissues. To date, PBPK models have been successfully developed for more than 40 compounds, including benzene.

PBPK models can be used to obtain more accurate estimates of cancer risks by using tissue doses to describe dose-response relationships rather than external levels of exposure to the parent compound. If nonlinear (saturable) kinetic processes are involved in metabolite production, the relationship between exposure and tissue dose can be nonlinear. Thus, it is possible that extrapolations of risk from high to low doses will be more accurate using tissue dose rather than external exposure, particularly when tissue response is proportional to tissue dose.

Pharmacokinetic modeling can also be useful in identifying multiple metabolites that may be involved in cancer induction. By identifying which metabolites of benzene are associated with cancer induction in animals and humans, it will be possible to identify the most appropriate measure of tissue dose for modeling purposes.

Pharmacokinetic models can also be used to evaluate the effects of multiple exposures that may compete for common metabolic pathways, thereby resulting in saturation of metabolism of critical pathways. Methylene chloride is known to be metabolized by two primary pathways, one of which is saturable and thus affects the rate of metabolism by the second pathway. Secondary metabolism of metabolites produced by the same compound may also be competitive; this occurs, for example, in the metabolism of trichloroethylene.

PBPK models are also useful in extrapolating between different routes of exposure and between different species. Route-to-route extrapolation is done simply by allowing for different routes of uptake within the PBPK model. Extrapolation between species is done by substituting appropriate physiologic and biochemical parameters for the target species into the PBPK model; in some cases, metabolic data may be obtained directly in humans, thereby avoiding the need to scale metabolic parameters across species.

Molecular Dosimetry

Direct measurement of tumor response rates at low doses is not possible, so extrapolation of cancer risks from high to low doses necessarily requires that assumptions be made about the shape of the dose-response curve at low doses. For DNA-reactive carcinogens, it is generally assumed that cancer risk is linearly related to dose within the low-dose region. Although several different statistical methods for linear extrapolation are available, extrapolation methods that impose linearity at low doses generally lead to comparable estimates of risk.

Current analytical methods for measuring DNA adducts in target tissues are highly sensitive, capable of detecting a single adduct in up to 10^8 nucleotides. Such advances in molecular dosimetry may provide an opportunity for direct estimation of low-dose cancer risks, if specific adducts associated with cancer induction can be identified and measured. For example, DNA-protein cross-links induced by formaldehyde may be associated with neoplastic transformation and may prove to be of use in low-dose cancer risk assessment for formaldehyde.

Non-DNA-Reactive Carcinogens

The guidelines for cancer risk assessment published by the U.S. Environmental Protection Agency (1986) are based on a default assumption of low-dose linearity for risk assessment purposes, in the absence of information to the contrary. If the mechanism of action of specific carcinogens is known, estimates of carcinogenic risk may be based on extrapolation methods that are nonlinear at low doses.

Carcinogens that induce cancer without direct interaction with genetic material may exhibit

qualitatively different behavior at low doses than DNA-reactive carcinogens. Suppose, for example, that a non-DNA-reactive carcinogen were known to exert its effects as a consequence of toxic injury to the target tissue, resulting in cell proliferation and increased cancer risk. If toxicity occurred only at high doses, such an agent may present little or no carcinogenic risk at low doses.

Time-Dependent Exposures

In many cases, the level of exposure to a toxic substance will not be constant over time. Since the relative effectiveness of exposures occurring at different ages will vary, it is important to take temporal variation in exposure levels into account in risk assessment. The effects of peak exposures occurring during relatively short time intervals may also produce different risks than the same cumulative exposure experienced at a constant rate over the same time period.

The extension of BBDR models for carcinogenic effects to incorporate time-dependent exposure patterns is technically straightforward. However, relatively few experiments with chemical carcinogens have been conducted using time-dependent exposure patterns. Such experiments are necessary to validate projections of risk based on BBDR models for different exposure patterns.

Integrated Pharmacokinetic and Pharmacodynamic Risk Assessment Models

A comprehensive model for carcinogenic risk assessment requires integration of pharmacokinetic (PK) models for tissue dosimetry and pharmacodynamic (PD) models for tissue interaction. Integrated PK and PD models have recently been developed for TCDD, including a PBPK model for metabolism, liver enzyme induction based on binding to the Ah receptor, and a two-stage clonal expansion model for neoplastic transformation of hepatocytes. The development of such models not only provides as complete a description as possible of carcinogenic risk, but also provides opportunities for identifying knowledge gaps in risk assessment, as well as a means for identifying critical parameters that may lead to uncertainty in risk characterization.

Characterization of Uncertainty

Many factors contribute to uncertainty in estimates of carcinogenic risk. A recent trend in risk assessment involves the development of distributions of uncertainty, taking into account as many sources of error as possible. For example, uncertainty in the values of the parameters involved in PBPK models will contribute uncertainty to predictions of concentrations of reactive metabolites in target tissues. Portier and Kaplan (1989) used estimates of uncertainty in allometric, biochemical, and metabolic parameters in a PBPK model for methylene chloride within the context of an integrated pharmacokinetic/pharmacodynamic model to develop a distribution of uncertainty for the dose estimated to cause a lifetime cancer risk of one in a million. Other factors such as the level of exposure can also be included in uncertainty analyses.

Carcinogenic Mixtures

Humans are subject to both multiple exposures and exposure to complex mixtures such as polycyclic aromatic hydrocarbons (PAHs). Laboratory studies have indicated that joint exposure to two carcinogens can lead to synergism, antagonism, or no interaction (see Krewski and Thomas 1992); synergism has been observed in epidemiologic studies involving joint exposure to different agents (National Research Council 1988). Although interactive effects may not be apparent at low levels of exposure (Kodell et al. 1991), the possibility of synergism should be considered whenever joint exposure to two or more agents occurs.

Different approaches have been proposed for assessing the risks associated with complex mixtures (National Research Council 1988). With PAHs, for example, the use of a single marker compound such as benzo[a]pyrene could be considered, but mixtures of PAHs are sufficiently variable in composition that the potency of the mixture cannot be characterized by a single component. Relative potency factors already established for several PAHs can be used to obtain more accurate estimates of the risk associated with mixtures of PAHs, although this requires that the composition of the mixtures be known, that the potency of the mixture is essentially determined by the PAHs for which relative potency values are

available, and that there is no interaction among the components.

Other methods for assessing the risks of complex mixtures have been proposed, including bioassay directed fractionation to identify the biologically active components of the mixture. Further research on the utility of these and other methods for assessing the carcinogenic risks of complex mixtures would be helpful in identifying the most appropriate methods to be used with mixtures of air toxics.

Risk Assessment for Noncarcinogenic Effects

Quantitative risk assessments for carcinogenic and noncarcinogenic effects proceed along quite different lines. In the absence of information to the contrary, carcinogenic risk assessment is based on the assumption that the dose-response curve is linear in the low-dose region. For most noncarcinogenic effects, a threshold is presumed to exist, and a reference dose is established by applying a suitable uncertainty factor to the no-observed-adverse-effect level (NOAEL).

Crump (1984) introduced the concept of a benchmark dose (BMD) for noncarcinogenic risk assessment. The BMD is defined as the dose leading to an extra risk (such as 5% to 25%) within the observable response range. The BMD is a more precisely defined quantity than the NOAEL and has been recommended by the U.S. Environmental Protection Agency (1991b) as an adjunct to the traditional safety factor method in developmental toxicity risk assessment. Ryan (1992) describes the calculation of the BMD for developmental toxicity data. Gaylor and Slikker (1990) consider the case of risk assessment for neurotoxic effects.

The BMD may ultimately provide a way to unify carcinogenic and noncarcinogenic risk assessment. Specifically, the BMD evaluated near the lower limit of the observable response range (say an excess risk of 5% to 10%) could be used as the starting point for quantitative risk assessment in either case. A suitable system of safety factors could be established for noncarcinogens and carcinogens; in the latter case, the application of a safety factor to the BMD would be effectively equivalent to linear extrapolation from the risk at which the BMD is evaluated.

MOLECULAR BIOLOGY

It may be taken as an article of faith that increased knowledge of mechanisms has a positive impact on the validity and precision of risk assessment models and procedures. Molecular biology is a scientific discipline that offers a set of conceptual and methodologic tools which are extremely useful when applied to questions of basic mechanisms. For example, chemicals that act via genotoxic or mutational mechanisms would clearly be subjected to a different risk assessment model than would chemicals whose mechanism is largely promotional or cytotoxic. Although this point is not novel, it turns out that in many instances molecular experiments have shed new light on the problem. A good example is formaldehyde, an agent whose nonlinear dose response and cytotoxic effects might be taken to indicate a nongenotoxic mechanism of carcinogenesis. The recent discovery of p53 gene mutations in formaldehyde-induced tumors suggests the possible existence of a genetic component important to the action of this agent. Similar data were developed for the putative nongenotoxic carcinogen furan, which produced liver tumors with a distinct pattern of mutations activating the *ras* gene.

Molecular biological approaches to the study of chemicals or classes of chemicals under consideration have a variety of potential contributions to make in improving our knowledge of health effects and risk assessment. This potential cuts across the list of specific chemicals and may be applied to any one or to all. This is partly because the state of current knowledge related to these five categories ranges from none to very little. Three broad areas that were touched upon in the reports of four of the five working groups (methanol being the exception) will be discussed here; these are carcinogen-specific effects on cancer genes (oncogenes and tumor suppressor genes), comparison of interspecies mechanisms, and molecular markers of exposure or dose.

Since the pioneering work of the Millers (Miller and Miller 1947, 1966) and others several decades ago, it has been generally accepted that chemical carcinogens exert their effects through interaction with the genetic material—DNA. Although there are exceptions to this idea (the nongenotoxic carcinogens, for example), we have strong evidence that cancer is a consequence of

somatic mutations in target cells. Until the early 1980s the focus of research into mechanisms of chemical carcinogenesis was on the general biological or genetic effects as determined by DNA binding, damage, and repair, or by mutations in marker genes. Knowledge of specific genetic loci that could serve as targets for the action of chemical carcinogens was nil, as was understanding of the mechanistic basis for translation of genetic damage into cell transformation. The situation changed dramatically after the discovery of mutationally activated cellular oncogenes such as the *ras* gene family. For the first time hypotheses could be generated to explain the molecular mechanisms by which a reactive chemical carcinogen would, for example, bind to the O⁶ position of guanine at codon 12 of the *H-ras* gene and produce a DNA adduct, which results in a G-to-A transition mutation, which in turn activates that particular gene from a protooncogene to an oncogene, causing an initiating event and beginning the long, complex process of malignant transformation.

Early work on the role of oncogenes in chemical carcinogenesis from a number of laboratories raised the hope that particular chemicals might exhibit signature patterns of oncogene activation in the tumors they induced in animal models. Although evidence for carcinogen specificity was obtained in certain model systems, other experimental evidence made it clear that tissue and species specificity were at least of equal importance in determining the particular molecular pathway involved in any experimental tumor model (Garte 1987). To make matters even more difficult, it soon became clear that even in a specific model such as rat mammary carcinomas induced by *N*-methylnitrosourea (NMU), specific oncogenes were never found to be activated in 100% of the cases (Zarbl et al. 1985). In other words, multiple molecular pathways exist in all tumorigenesis models, each involving different genes at different frequencies. More current research, including the exciting findings of tumor suppressor genes in sporadic human cancers, are completely consistent with this paradigm. Except for the rare inheritable disease retinoblastoma, in which every case involves a defect in the function of the *Rb* gene, there are no models of cancer that exhibit unequivocally consistent patterns of tumor suppressor gene or oncogene involvement.

However, the importance of the p53 tumor suppressor gene in many types of human cancer has once again raised the possibility of detecting carcinogen-specific fingerprints at the molecular level. Unlike *ras* activation, which requires a mutation at one of only six possible sites, inactivation of the p53 suppressor gene may occur at a large number of sites. Work on aflatoxin-induced human liver cancer from China has, in fact, demonstrated that a specific mutational site in this gene could serve as a marker for a specific chemical etiology of the disease (Hsu et al. 1991). Clearly, more research needs to be done to further explore this approach.

When discussing molecular biological issues across chemical lines, to include agents as diverse as formaldehyde, benzene, butadiene, and aromatic hydrocarbons, it is essential to address the issue of carcinogen-specific effects on specific and relevant target genes. This issue was raised in several of the individual working papers, along with the related question of whether a molecular endpoint detected in a rodent tumor model could be useful in the difficult problem of interspecies extrapolation for human risk assessment. For example, if it were found that rat tumors of a particular tissue induced by agent X contain a high frequency of p53 genes inactivated by a G-to-T point mutation at codon 247, could one then attribute the cause of human tumors of the analogous type showing the same p53 mutation to exposure to agent X?

Unfortunately, current data are not encouraging in this regard. There are many examples of divergent molecular pathways between human and rodent tumors. For example, human prostate tumors rarely if ever show evidence of *ras* gene activation whereas experimentally induced rat prostate tumors exhibit a significant incidence of H-*ras* activation (Sukumar et al. 1991). Furthermore, the high frequency of p53 gene mutations in human tumors of diverse tissues is not reflected in many rodent tumor models. In fact, the finding of p53 mutations in formaldehyde-induced rat nasal tumors, as described in the report on aldehydes, is a rare case of a rat tumor model shown to have tumor suppressor gene involvement.

It is possible that the lack of correlation of molecular endpoints across species is due to basic differences in the mechanisms of carcinogenesis between rodents and humans. If this turns out to be the case, then human cancer risk assessment

based on mechanistic data from animal experiments would be severely limited on theoretical grounds. However, an alternative view, which should provide a fertile field for research, is that since the inducing agents for most human tumors are not known, differences between human and rodent gene activation profiles are due to differences in the chemical (or other) etiologic factors. These issues are amenable to experimental investigations. A successful resolution of these questions will require cooperative efforts between molecular biologists, epidemiologists, clinicians, and scientists involved in exposure assessment and dosimetry.

The issue of carcinogen dose as applied to oncogene or tumor suppressor genes has hardly been mentioned in the literature of molecular carcinogenesis. Dose-response effects of carcinogens on oncogene activation have rarely, if ever, been seen in end-stage tumors. When activation of a particular oncogene is a requirement for tumorigenesis in a particular model, dose-response effects on gene activation in the end-stage tumor will not be observable because selection pressures require that every tumor, regardless of the original dose effect on the frequency of oncogene activation in the target tissue, must contain an activated gene. However, if a gene is not required for tumorigenesis, either because its activation does not contribute to any carcinogenic mechanisms in that tissue, or because alternative mechanistic pathways involving other genes exist, then observation of a dose-response effect in end-stage tumors is possible (Felber et al. 1992).

In contrast to the situation for effects on selectable gene function, other molecular endpoints have been efficiently and successfully used as internal dosimeters for exposure and dose-response studies. These include DNA adducts, DNA-protein cross links (as exemplified by formaldehyde), and the level of expression of inducible genes. The development and validation of new molecular biomarkers that may serve as qualitative and quantitative determinants of exposure to specific agents or classes of agents holds considerable promise for advancing the scientific basis of risk assessment from the exposure side. Here again, interspecies extrapolation may be problematic (although probably less so than in cancer gene studies). This points to the desirability of developing assays that

can utilize human surrogate sources of biological material such as blood cells, hair, and urine. This field of molecular human epidemiology is still in its infancy, and although there is tremendous potential, a great deal of theoretical as well as experimental work must be done in order to begin to use molecular biomarker data in risk assessment procedures with confidence.

The molecular biological approach can be applied to case control types of situations as well as to the standard exposed cohort studies that are often used to determine the carcinogenicity of specific single agents. For example, biological samples (tumor tissue, blood) from cases of lung or liver cancer among populations known to have high levels of exposure to agents such as radiation or aflatoxin may be used to determine whether specific endpoints in cancer genes or other markers are potentially predictive for that disease caused by the particular agent (Groopman et al. 1992).

The process of carcinogenesis is multistage and dynamic. Evidence indicates that early benign lesions progress to lethal metastatic cancers through a process of cellular evolution enhanced by selective pressures and driven by increasing genetic instability as manifested by gene amplification, chromosomal aberrations, and other mutations. Exposure to chemicals of all types could impact on the progression of tumors by directly affecting mutation rates or exerting selective pressures (through toxic effects, for example) on clones of tumor cells. Research into the molecular mechanisms of tumor progression is just starting, and information on the effects of chemical pollutants on this process barely exists at all. However, it may be safely assumed that the role of these agents in human carcinogenesis is not strictly limited to initiation or the earliest stages. Molecular biological approaches can help to elucidate the effects of exposure of such agents on the entire complex and dynamic process of cancer induction and progression to lethality in human beings.

Research Recommendations.

- *Strengthen the theoretical bases for human molecular epidemiology by developing a uniform statistical and methodologic approach to treatment of data from diverse populations using a variety of endpoints.*

- *Develop and validate new molecular biomarkers for use in exposure assessment, biologically effective dosimetry, and adverse health effects.*
- *Explore genetic markers of susceptibility and sources of individual variation in human responses to toxics.*
- *Identify molecular alterations at loci such as ras, p53, and Rb in human cancers of defined chemical etiology (where possible) for comparison with results from animal experiments.*

The first recommendation can probably be accomplished within 3 years or sooner, and is required for the success of the second and third recommendations. These goals should produce useful results in the form of new assays by the end of 3 years, but would also be ongoing areas of research. The last recommendation can be anticipated to require more than 3 years of research to achieve useful mechanistic data for risk assessment.

EPIDEMIOLOGY

A goal of epidemiologic studies should be to establish quantitative exposure-dose-response relationships. While this goal may not be attainable, several important pieces of information may be obtained from the human data base, in particular to determine some bounds on the human potency of the agent.

The chronic disease outcomes associated with hazardous chemical exposures are rare events often with a long latency period (i.e., time from first exposure to onset of disease). Cancer, for example, usually does not occur until at least ten years after initial exposure. Therefore, it is generally not feasible to directly follow exposed and unexposed populations for the risks of cancer. Studies of disease resulting from past exposures to a toxic chemical can provide a substitute for the prospective multiyear follow-up of exposed individuals. Most human studies used for risk assessment come from the repeated exposures in occupational settings. The ideal study would be one in which individuals with documented exposures to a toxic substance in the general environment are followed to determine the incidence of cancer or other diseases. These

studies are probably more feasible for acute effects or diseases with short latency periods.

Issues in the Use of Human Data to Assess Risks

In the absence of human studies, human risks associated with chemical exposures have been determined from models that extrapolate disease rates in animals. When human studies have been available, they have usually been limited to selected populations exposed occupationally or accidentally to high doses of a toxic agent relative to the usual low levels in the general environment. These historical or retrospective studies have both problems and advantages. Because they are usually conducted in occupational settings where the exposures are higher than in the general environment some of the same issues regarding extrapolation from high to low doses which plague risk assessment from animal data are pertinent to these studies as well. In addition, exposure levels have usually not been documented over the study period from which cases are taken. Diseases are identified through mortality records, and the accuracy and completeness of the information is often questionable. Finally, there is usually little information available regarding potential confounding variables or other exposures that could influence the disease risk. Despite these limitations, occupational studies are useful for risk assessment because workers may have high exposures, which should result in a greater risk of disease than in the general population. However, these working populations all have special characteristics that must be kept in mind when interpreting risk data. They do not represent the usual environmental population distribution in terms of age, race, sex and other demographic characteristics. Those subsets of the population with increased susceptibility because of underlying diseases may not be hired by industry. Some of these differences include the presence of other chemicals, the rigors of strenuous labor, and the variation in routes of entry into the body.

Issues in the Use of Animal Studies to Assess Risk

The use of animal models for risk assessment has been discussed extensively. The basic questions that have been raised are whether the animal metabolizes the chemical in the same way as the human, whether the tissue dose of the agent is similar in animal and man, and whether other characteristics of the animals such as the presence

of retroviruses may change species susceptibility. The other issue regarding the use of animal models is whether the practice of exposing the animals to high doses to obtain the maximum number of responses in a reasonable time frame can produce results that reflect low dose environmental exposures. Questions arise as to which mathematical model should be used for the low dose extrapolation.

Other factors must be considered when extrapolating animal data to the free-living human population. The exposed individuals may vary in susceptibility. Differences may be due to physiologic factors such as age and gender. Variation in response will occur due to life-style factors such as diet, smoking, prescription drug use, and alcohol intake. Still other differences may relate to hereditary factors such as variation in individual enzyme profiles or DNA repair. If the agent produces toxic effects other than cancer, such as reproductive losses, neurologic changes, cardiovascular or respiratory disease, the specific metabolic products related to these effects, the interaction of these products with the tissues, and the observed pathological changes may differ from those related to a cancer outcome. The physiologic, life-style, and hereditary factors that influence the susceptibility of individuals to these diseases may differ from those related to cancer. Some of these outcomes may be difficult to measure in animal systems.

Role of Epidemiologic Data in Risk Estimation

Studies in humans may not be able to establish the exact exposure associated with the disease outcome, but often they can identify an exposed worker population in which to determine whether or not the outcome occurs with greater than expected frequency. This is a first step to determining whether there is any evidence that human populations develop similar diseases to those found in animal species. The exposure scenario can be examined in these workers to determine whether they are exposed to multiple chemicals and whether the exposure is intermittent or continuous as well as other exposure characteristics. These exposure scenarios then can be tested in the animal models.

Despite the fact that we cannot establish all the steps in the pathway from exposure to resultant disease in an epidemiologic study, we may be able

to characterize how humans metabolize the chemicals using biologic fluids from occupationally exposed groups. We can also examine tissue fluids for DNA adducts to verify the presence of internal tissue exposure and the resultant chemical interaction with DNA. Finally, examination of mutation frequency as in the hprt gene, while not establishing a cancer pathway, would at least indicate that human exposure causes permanent changes in DNA.

These molecular epidemiology studies would subsequently need to be expanded to determine the variability in response of populations and to examine the characteristics of individuals that influence the variability in these endpoints. The characterization of populations in regard to the physiologic, life-style and hereditary factors that may determine differences in metabolism, DNA adduct formation rates and mutation rates would eventually allow the estimation of the number of individuals who might be at highest risk from exposure.

These studies to establish appropriate biologically based risk in humans and animals require many years to complete but eventually should allow determination of the appropriate method for extrapolating animal data to humans using physiologically based risk models.

Epidemiology of Air Toxics

For the potentially hazardous chemicals in mobile source emissions that are the subject of this report, the available information on human risk differs widely. For almost all of the exposures considered, with the exception of methanol, cancer has been the exposure end point of interest both in animal studies and in reports of human exposures in occupational settings. For these chemicals, little is known about the chronic effects of low level exposures on other outcomes such as reproductive, neurologic, respiratory, and cardiovascular effects. Virtually nothing is known about how individual variations in susceptibility may influence the effects of these chemicals.

For all the agents discussed in this report, information on the metabolism, tissue dose, and biological changes resulting from human exposure is inadequate. This information would permit the establishment of a physiologically based risk estimate to extrapolate animal data to humans. Various steps in the human side of that modeling are missing for most of the agents examined.

Further data are needed on the metabolism of the chemicals, their deposition in the body, the target tissues of damage, the ability to repair such damage, and the resultant frequency of mutation which may represent the initial steps to cancer and other disease. Chromosome aberrations and breaks, DNA adduct formation, or DNA-protein crosslinks have been reported for several of the agents, but details regarding the development of these changes have not been clarified. The interaction between other human conditions or diseases and the risk posed by exposure to the chemicals has not been delineated. For example, information is needed on the role of folate deficiency in the neurotoxic effects of methanol, the role of aplastic anemia as a requisite precursor to leukemia from benzene exposure, and the role of DNA repair in reducing mutation and cancer risk.

Exposure assessment in the general population is also an issue that needs to be addressed. Most human data used for risk assessment are obtained from populations of workers exposed in an industrial setting. The extrapolation of these occupational exposure measurements to those in the general environment represents a challenge. For polycyclic organic matter the problem is compounded because the mixtures and the particulates in all occupational settings studied may differ from any environmental situation. For some of the volatile chemicals the issue of where the measurement is taken can be a problem. Chemicals measured at the tailpipe may quickly disperse or react in the atmosphere so that the exposure to the population may not correspond to that in the exhaust. Yet tailpipe measurements are easier to standardize than measures taken in the general environment. In addition, for materials that can come from many environmental sources, the source of materials measured in air may be difficult to identify. Individuals are not exposed to the emissions directly at the end of the tailpipe but that may be the only point at which one can determine the contribution of gasoline versus other sources to the background level in ambient air.

Research Recommendations.

- *For all agents it is necessary to determine whether there are health effects in humans. While cancer has been the primary endpoint in epidemiologic studies of air toxics, non-cancer health outcomes may be more appropriate for*

questions regarding environmental exposures.

These studies may not be able to identify the exposure associated with the risk or to include all potentially susceptible populations, but they are the first step in establishing whether humans are affected at some level of exposure. These studies usually will be done in occupational populations. In some cases, the information may be derived from a further examination of the existing epidemiologic data or continuous follow-up of workers, as was recommended for benzene and butadiene.

- *For all agents there is a need to develop further data regarding the biological effects in humans.* This includes gathering information on metabolism, distribution of the material in target tissues, and signs of early damage to target organs. Thus, biological markers for both exposure and effect are needed. Gaps in the process of development of a physiologically-based risk assessment model for each of the agents need to be filled.
- *There is a need to identify populations whose exposures more closely represent those experienced by the general population and individuals who are likely to be more sensitive to the agent.* Determining the differences between the exposure circumstances in the general population versus the occupational setting may reveal major discrepancies between the type of mixture in one setting compared with the other.
- *Possible confounding variables in studies to establish a risk need to be identified in order to determine whether any observed risk in an occupational setting is due to the presence of the chemical of interest, or to personal characteristics of the individual, or other occupational exposures that are related to the disease.* The concurrent occurrence of two risk factors may play an important role in enhancing the risk. Therefore, the presence of the second factor must be examined as a potential interacting agent or condition that may multiply risk. These additional risk factors in the general population may be different from those observed in occupational settings.

Further studies in each of these areas are needed for all the chemicals discussed in this report. However, the amount of information existing in each of the areas differs for each of the chemicals. Therefore, priorities as to which areas to address first may differ for each chemical.

NEUROTOXIC, REPRODUCTIVE, AND DEVELOPMENTAL EFFECTS

Reproductive failure, birth defects, and central nervous system disorders are major societal problems. Estimates indicate that nearly one in five couples are sterile and that about 15% of recognized pregnancies end in spontaneous abortions (Warburton and Fraser 1964). Major congenital malformations are recognized during the first year of life in approximately 3% of liveborn infants, and 20% of postnatal deaths are due to birth defects (National Foundation/March of Dimes 1981). The causal factor in the majority of birth defects is unknown. Nervous system disorders are present in nearly 12% of children under 18 in the United States (National Academy of Sciences 1989), and neurotoxic disorders are one of the 10 leading causes of work-related disease and injury in the United States (National Institute for Occupational Safety and Health 1986).

The reproductive system, the developing fetus, and the developing or mature nervous system can be targets of occupational and environmental chemicals. Human reproductive dysfunction has been associated with exposure to industrial chemicals such as lead, vinyl chloride, and carbon disulfide, while many other chemicals have been shown to cause reproductive effects in animal models (Barlow and Sullivan 1982; Nisbet and Karch 1983). Industrial chemicals that are known human teratogens include polychlorinated biphenyls, mercury, and lead (Rees et al., 1990). Alterations in central nervous system function has been reported after inhaled exposure to certain industrial chemicals or chemical mixtures (toluene, xylene, gasoline). These effects can be acute or prolonged depending on the amount and duration of exposure (Burbacher 1993).

Effects of Air Toxics

Some of the mobile air pollutants under review at this workshop have been shown to affect reproduction and to be teratogenic or neurotoxic. The importance of these endpoints in comparison to other effects such as cancer, however, depends on the individual chemical. A comparison across different endpoints is essential for determining priorities for research aimed at reducing the uncertainty in risk assessment. Endpoints which can be reliably demonstrated at low levels of

exposure should be prioritized. A comparison of different endpoints for the chemicals under review follows.

Benzene has been shown to affect fetal development and central nervous system function. These effects include a reduction in fetal weight and skeletal changes, observed in rats following maternal inhalation exposure (Green et al. 1978); neurobehavioral effects, reported in adult mice following approximately 1 week of inhalation exposure (Dempster et al. 1984); or effects on motor activity and response to d-amphetamine challenge, reported in rats following neonatal benzene exposure (Tilson et al. 1980). While the above effects are important for defining benzene toxicity, the effects are observed at exposure levels well above those associated with hematological changes. Thus, the data to date indicate that the primary effect of benzene exposure is leukemia.

The marked species difference between mice and rats in the carcinogenicity of 1,3-butadiene is also observed for teratogenic effects of this chemical (Hackett et al. 1987a and 1987b). In mice, exposure to 1,3-butadiene on gestational days 6-15 has been related to a decrease in maternal, fetal and placental weights at the lowest exposure concentration studied. By contrast, exposure to rats at the same concentrations does not produce effects on the fetus. Reproductive toxicity has also been reported in mice following 1,3-butadiene exposure. Ovarian atrophy was observed at the lowest exposure studied; while testicular atrophy was reported at higher exposure concentrations (Melnick et al. 1990). These teratogenic and reproductive effects have been observed at levels of exposure comparable to those associated with carcinogenesis. These effects may be important endpoints to consider in assessing the risk of human exposure to 1,3-butadiene.

Visual and central nervous system toxicity are the primary effects associated with methanol exposure (Jacobsen and McMartin, 1986). Exposure to methanol in utero has been shown to cause fetotoxicity (Rogers et al. 1992). Significant increases in the incidence of embryo/fetal death, exencephaly, cleft palate and skeletal anomalies, and reduced fetal weight have been reported. While there are marked species differences in the fetotoxic effects of methanol, rats do not show the effects discussed above (Nelson et al. 1985), it is likely that fetotoxic endpoints will be important in

determining the risk associated with human methanol exposure.

Many of the reported effects associated with chronic low-level exposure to aldehydes include nervous system disorders. In addition to irritant effects, dizziness, headache, lack of ability to concentrate, and memory loss are all common symptoms related to low level exposure to formaldehyde (Breysse 1988). Acetaldehyde has been implicated in studies of the reproductive and neuroteratogenic effects of ethanol (Mulvihill and Yeager 1976). In addition, direct exposure to acetaldehyde has been associated with defects in the developing brain following in utero exposure (O'Shea and Kaufman 1976). While the significance of these findings at levels of exposure that are associated with irritant effects is unknown, additional studies of the neurotoxic and teratogenic effects of chronic low-level aldehyde exposure should be pursued. It is likely that endpoints associated with nervous system effects will be important in determining the risk associated with human exposure to aldehydes.

Conclusions

It is impossible to determine with certainty the degree to which chemical exposure is associated with the problems outlined in the initial paragraph. Many chemicals have been shown to cause reproductive, developmental, or neurotoxic effects in controlled laboratory studies using animal models. Others have been shown to cause effects in humans at relatively high levels of exposure. The key question that has yet to be answered for most of these chemicals is "what is the risk to humans of chronic, low-level exposure at critical periods in development or over a lifetime?" While this question is a difficult one to address, the magnitude of the problem requires that we consider it carefully in our deliberations on how best to reduce the uncertainties in assessing the risks associated with human exposure to these chemicals.

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HEI

**THE HEALTH EFFECTS INSTITUTE
MOBILE AIR TOXICS WORKSHOP
RESEARCH PRIORITIES TO REDUCE
UNCERTAINTIES IN RISK ASSESSMENT**

Workshop Program

December 4-6, 1992
Hyatt Regency Monterey

Monterey
California

THE HEALTH EFFECTS INSTITUTE
MOBILE AIR TOXICS WORKSHOP
RESEARCH PRIORITIES TO REDUCE UNCERTAINTIES IN RISK ASSESSMENT
1992

Friday, December 4

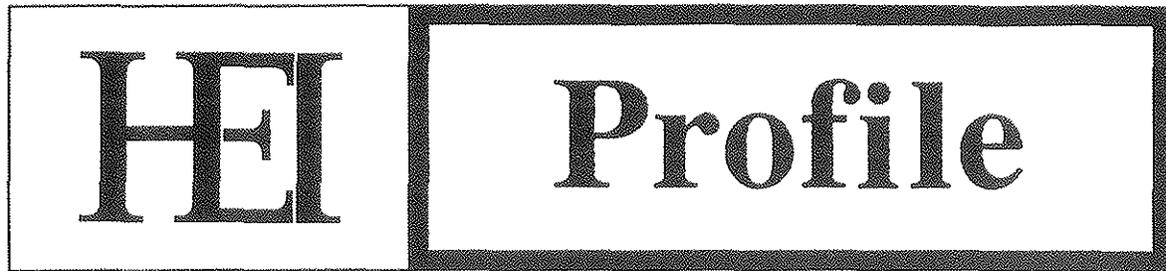
- 2:30-2:45 pm **Welcome**
Charles W. Powers, Acting President, HEI
Bernard Goldstein, Environmental and Occupational Health Sciences
Institute, Chairman, HEI Research Committee
- 2:45-3:45 pm **Discussion of the regulatory context**
Chair: *Roger O. McClellan*, Chemical Industry Institute of Toxicology
Discussants: *Charles Gray*, *Phil Lorang* and *Judith Graham*, U.S. EPA;
Carol Henry, California EPA; *Michael Scheible*, California Air Resources
Board
- 3:45-4:00 pm **Charge to the working groups**
Bernard Goldstein
- 4:15-6:30 pm **Concurrent working group sessions**
Chairs: *Roy Albert*, University of Cincinnati (butadiene); *Rory Conolly*,
Chemical Industry Institute of Toxicology (aldehydes); *George Lucier*,
National Institute of Environmental Health Sciences (benzene); *Joe*
Mauderly (POM); *Bernard Weiss*, University of Rochester (methanol)
- 7:00-8:00 pm **Dinner**
- 8:00-9:00 pm **Keynote address**
Erich Bretthauer, Assistant Administrator for Research and Development,
U.S. EPA

Saturday, December 5

- 8:30-11:00 am Concurrent working group sessions
- 11:30-1:00 pm Working group presentations
Chair: *Bernard Goldstein*
- 1:00-5:00 pm Lunch and free time or individual writing
- 5:00-7:00 pm Concurrent working group sessions
- 7:00-8:00 pm Dinner
- 8:00-9:30 pm Panel discussion: Establishing priorities for regulation of fuel and technology
Chair: *John Moore*, Institute for Evaluating Health Risks
Discussants: *Donald Buist*, Ford Motor Co.; *Charles Gray* and *Lawrence Reiter*, U.S. EPA; *John Holmes* and *Michael Scheible*, California Air Resources Board; *Carl Mackerer*, Mobil Oil Corp.; *Robert F. Sawyer*, University of California at Berkeley; *Harold Wimette*, Chrysler Corp.

Sunday, December 6

- 8:30-10:00 am Working group presentations
Chair: *Bernard Goldstein*
- 10:00-1:00 pm Concurrent working group sessions
- 1:00-2:00 pm Lunch
- 2:00-3:00 pm Discussion session of: Working Group chairs, rapporteurs, experts on cross-cutting issues
- 3:00 pm Adjourn


 The graphic features the letters 'HEI' in a large, bold, serif font on the left, enclosed in a thin black border. To its right, the word 'Profile' is written in a large, elegant serif font, also enclosed in a thin black border. The two elements are set against a white background with a dark, textured border around the entire graphic.

HEI Profile

THE HEALTH EFFECTS INSTITUTE

What is the Health Effects Institute?

In 1970, the Clean Air Act set the stage for a decade of confrontation between the Environmental Protection Agency (EPA) and the automobile industry. The debate over implementing the Act's emission standards was particularly fractious. In 1977, new amendments to the original Act required certification by manufacturers that a vehicle's emissions did not "cause or contribute to an unreasonable risk to public health, welfare, or safety" and granted EPA the authority to require appropriate testing. Out of the ensuing turmoil and uncertainty, the Health Effects Institute (HEI) was born.

The Institute's purpose was to provide the scientific foundation that the EPA, the motor vehicle and engine manufacturers, and the public needed for regulatory decision-making. The purpose was to develop the facts concerning health effects so carefully and credibly that controversy about the facts themselves would be removed from the adversarial agenda, and the debates over clean air could instead focus, properly, on national policy issues.

In 1980, HEI was chartered as an independent non-profit corporation "organized and operated . . . to support the conduct of, and to evaluate, research and testing related to the health effects of emissions from motor vehicles." And at the press conference where the Institute's founding was announced, then EPA Administrator, Douglas Costle, said that, "We anticipate that the Institute will become, if all goes well, the major source of studies on the health effects of motor vehicle emissions in the country."

The Institute's annual operating budget is contributed equally by the EPA and manufacturers and marketers of motor vehicles in the U.S. (although the Institute has funded several special projects with support from other sources as well). The automotive industry's share is contributed by 28 companies, most of whom are world-wide producers of automotive products; each company's contribution to HEI is, however, proportional to its U.S. vehicle and engine sales. The Institute has developed consultation processes with its sponsors and other public organizations to help focus its research priorities. However, none of the contributors has any control over the selection, conduct, or conclusions of HEI studies, and HEI makes no recommendations on how to apply research to regulatory and social policy. The Institute's autonomy is guaranteed, even beyond the statements in its charter, by the integrity and commitment of both its scientific leadership and its Board of Directors, which has been chaired by Archibald Cox since HEI's inception.

How Does the Health Effects Institute Work?

HEI is dedicated to first-rate science from definition to review. The projects that HEI funds either provide scientific information of direct and immediate regulatory relevance, or pave the way for developing such information in subsequent research. Two independent committees conduct HEI's scientific program. The committees themselves work in an environment uniquely structured to foster the necessary separation between research selection and oversight on the one hand and critical review of results on the other. This separation permits HEI to

both fund and guide research, as well as to provide credible peer review of that same research. A scientific and administrative staff works with the committees and conducts the Institute's business.

The Health Research Committee, chaired by Dr. Bernard Goldstein, is responsible for defining, executing, and overseeing the research agenda. The Research Committee and its staff seek advice from HEI's sponsors and from other interested parties to determine research priorities. Once the Research Committee has defined an area of inquiry, the Institute announces to the scientific community that research applications are being solicited. Applications are reviewed for scientific quality by a panel of experts, and then by the Research committee. Studies recommended by the Committee undergo final evaluation by the Board or Directors, which also reviews the procedures, independence, and quality of the selection process.

But HEI is more than an extramural research program funding good individual research projects. By working in a collaborative fashion with the principal investigators of individual projects within a specific sphere of inquiry, the Research Committee typically helps to build a research program designed to give more and better answers to issues of regulatory significance than would be the case were the individual studies carried out in the original form and without reference to related studies. During the course of each study, HEI monitors progress by means of periodic reports and site visits. When the study is completed and the investigator submits the final report, the Research Committee's involvement with the study ceases.

The Health Review Committee, chaired by Dr. Arthur Upton, is not involved with ongoing studies in any way. It and the Review staff assess the scientific quality of each study and evaluate the study's contribution to unresolved scientific questions in a stringent peer review process. Final reports, as published by HEI, consist of both the investigator's report and the Review Committee's commentary on that report. The Review Committee's commentary discusses the strengths and limitations of the study and sets the findings into a scientific and regulatory context. The Institute publishes a one page Statement which summarizes the report and its commentary and is written for non-scientists. (In addition, all HEI investigators are urged to publish the results of their work in peer-reviewed literature.) The Review Committee has

recently adopted a policy of publishing reports of related studies together in composite reports. These reports are intended to provide the same critical evaluation of individual studies while assuring that the relationship among studies is addressed in the overall commentary.

Where is the Health Effects Institute Now?

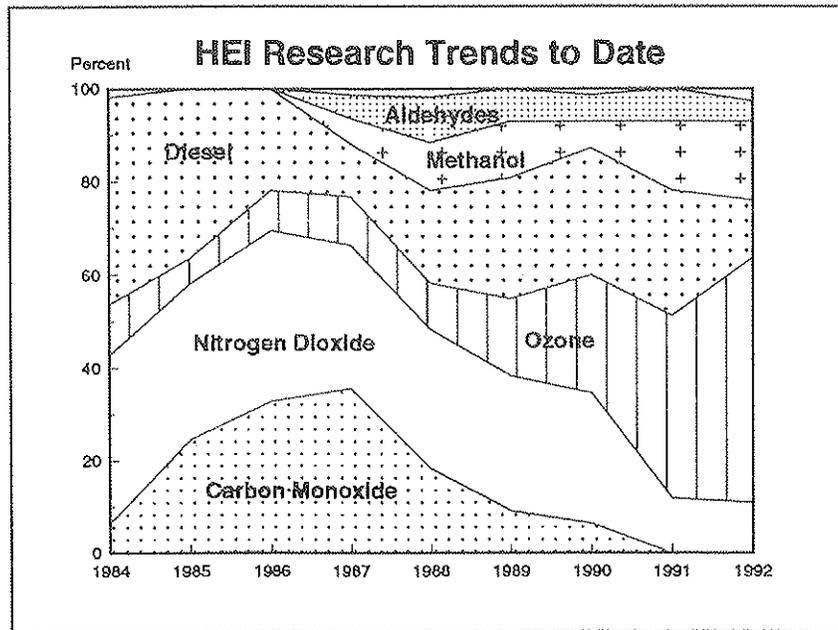
HEI is just twelve years old. Nevertheless, in that time, HEI has sponsored an impressive body of work (see its *Publications and Documents* booklet) and has been able to expand the community of first-rate scientists involved in environmental research.

HEI research has focused primarily on the following pollutants: components of diesel exhaust, oxidants (nitrogen dioxide and ozone), carbon monoxide, and methanol and aldehydes. Because, in 1980, substantial growth in the light-duty diesel vehicle fleet was expected, HEI's initial research sought better understanding of the health effects of diesel particulates and their adsorbed organic compounds. Currently, the Institute is completing a review of the scientific literature on the health effects of diesel exhaust. In light of this full evaluation, the Institute may pursue additional research on diesel issues.

HEI has supported many studies of carbon monoxide (CO) and oxides of nitrogen (particularly NO₂). Beginning with a variety of research approaches on each pollutant, HEI crystallized its work on each with a major human study. The Institute currently plans no additional studies on these pollutants because the questions that were identified have, to a large extent, been addressed.

Currently, ozone is a pollutant of major concern because it is not known whether chronic exposures to ambient ozone levels contribute to the development of lung disease. HEI's research program on the health effects of ozone includes chronic studies in conjunction with the National Toxicology Program, clinical studies of sensitivity to ozone, epidemiology, and ozone dosimetry.

New provisions in the 1990 Clean Air Act and concerns of the Institute's sponsors and other publics have recently led the Institute to focus on emerging issues where both new and reformulated fuels and automotive technology changes will result



in many additional questions about health effects of motor vehicle emissions. HEI is sponsoring important studies on the effects of methanol, and in December 1992, sponsored a major workshop to define the research needed for resolving key uncertainties that limit risk assessments of a variety of specific air toxics emitted by motor vehicles. The Institute will issue a report of that workshop in the Spring of 1993. The Research Committee is currently developing a 10-year strategic plan for an expanded research program that emphasizes these emerging scientific issues.

How Does the HEI Concept Apply to Other Environmental and Public Health Issues?

The fundamental concept on which HEI is based is that an independent Board of Directors governing an organization whose funding comes from government and regulated industries can recruit first-rate scientists to its Committees and staff to define, oversee, and review a coordinated extramural research program. Such a program will, in turn, draw good scientists and excellent institutions into work with regulatory significance.

The basic concept has been used in a spin-off from the original HEI. The Health Effects Institute—Asbestos Research was created in 1989.

A full range of scientific uncertainties arising from the presence of asbestos in buildings led Congress to request HEI to create and carry out a research program to address the diverse measurement, health significance, and remediation effectiveness issues. HEI-AR has published a widely-cited Literature Review of these issues and is carrying out a targeted research program to help resolve such remaining uncertainties.

The Institute is frequently approached about the possible application of the HEI concept to other issues. For example, it will, in the Spring 1993, release a major report on a specific research strategy to determine whether there are adverse health effects associated with certain electric magnetic fields. This work was supported equally by the EPA and several utilities companies.

The HEI Board, in the Fall of 1992, formed a Committee to explore organizational changes which would enhance the growth and replication of the HEI concept. This Committee is chaired by Dr. Donald Kennedy, a member of the boards of both HEI and HEI-AR. Charles W. Powers, the founding Executive Director of the Institute, has recently returned to serve as Chief Executive Officer of HEI and HEI-AR and to help initiate and oversee the transition being developed by the Kennedy Committee.



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The Health Effects Institute (HEI) is an independently governed nonprofit corporation founded in 1980 to provide objective, credible, high-quality information on the potential human health effects of motor vehicle emissions. HEI is funded equally by the U.S. Environmental Protection Agency (EPA) and 28 automotive manufacturers and marketers in the United States.

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