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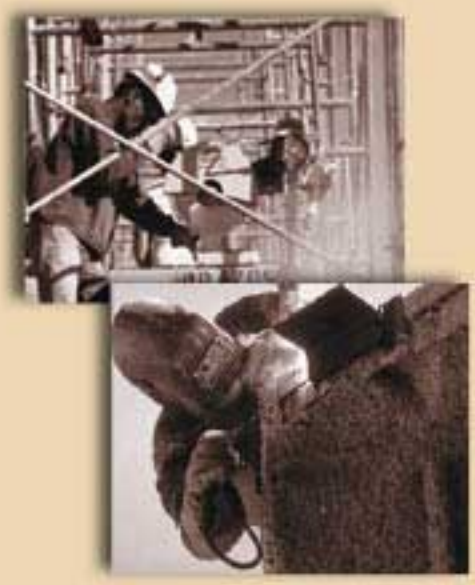
Mixed Exposures Research Agenda



A Report by the NORA Mixed Exposures Team

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

NIOSH

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Foreword Ê

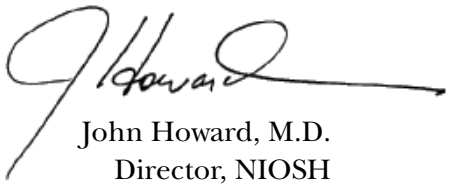
In April 1996, the National Institute for Occupational Safety and Health (NIOSH) and its partners unveiled the National Occupational Research Agenda (NORA). NORA was developed by NIOSH and more than 500 partners in the public and private sectors to provide a framework to guide occupational safety and health research into the next decade. This effort to guide and coordinate research for the entire occupational safety and health research community is focused on 21 priority areas. The areas are divided into three categories: (1) disease and injury, (2) work environment and workforce, and (3) research tools and approaches.

One of the identified NORA priority areas in the environment and workforce category is the study of mixed exposures. Combining government researchers and industry experts, a NORA Mixed Exposures Team was established to facilitate the study of occupational mixed exposures. Workers from agriculture, construction, mining and other industries are commonly exposed to combinations of chemical substances, biological or physical agents, and other stressors. Knowledge is limited of the potential health effects of mixed exposures. Additional nonwork-related exposures (such as the consumption of alcohol or tobacco or the use of insect repellents, cosmetics, or other chemicals) and individual susceptibility also add to the complexity of exposure and resulting biological responses. New approaches are needed to identify additive, synergistic, antagonistic, or potentiation effects from multiple exposures (sequential or simultaneous). Identifying these effects can help characterize worker exposure, conduct research at environmentally relevant levels, improve laboratory and statistical analysis methods, and develop hazard controls that take into account the components of the mixtures.

Research has shown that physiological interactions from mixed exposures can lead to an increase in severity of the harmful effect. For example, exposure to noise and the solvent toluene results in a higher risk of hearing loss than exposure to either stressor alone. Exposure to carbon monoxide and methylene chloride produces elevated levels of carboxyhemoglobin, reducing the blood's ability to carry oxygen in our bodies. The problem of mixed exposures is multifaceted, given the large number of combinations that occur every day in a variety of workplaces and in our everyday life experiences.

This report is the product of the NORA partnership team formed from experts inside and outside the public sector. The NORA Mixed Exposures Team examined the literature, cataloged ongoing research, and identified significant research gaps. Through examination of knowledge gaps and opportunities to leverage overlapping interests, the team identified key areas in which new research could significantly advance the science needed to develop future interventions. Those products, once implemented, could be used to reduce the risk of occupational disease and injury to workers.

The intent of this document is to articulate many of the issues involved with mixed exposures as well as to recommend research strategies and define research priorities that could lead to improved interventions for protecting workers from mixed exposures. We hope that this document will facilitate further dialogue about mixed exposures and generate keen interest among occupational safety and health researchers to devote attention to this important research area. In particular, we envision that this document could be used as the working paper for a future workshop on mixed exposure research needs and could help stimulate new outcome-focused research proposals. NIOSH will use the priorities outlined in this document (and refined through future workshops) as a tool for directing our internal research program, and for guiding our extramural activities.



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Contents

Foreword ii
Executive Summary vi
Abbreviations viii
Acknowledgments x

1 Introduction 1
Background 1
Scope 4

2 Analysis of Research Needs 6
Identifying Mixture Hazards 6
Effects Studies 10
Exposure Analyses 18
Biomarkers 21
Risk Assessment Methods 23
Controls 29

3 Priorities for a Research Agenda 32

References 34

Appendix 38

Executive Summary Ê

Workers are continuously exposed to a wide variety of chemical substances, biological agents, physical agents, and other stressors encountered both in and out of the workplace. Each stressor has the potential to cause a physiological effect, whether it is a prescribed pharmaceutical, consumed food, cleaning product, automotive exhaust emission, solvent, ultraviolet radiation, noise, whole-body vibration, or social or psychological stress. Mixed exposures may produce acute or chronic effects or a combination of acute and chronic effects, with or without latency. Other exposures in combination with certain stressors may produce increased or unexpected deleterious health effects, or they may combine or interact in the environment to create a new exposure risk. Exposures to mixed stressors can produce health consequences that are additive, synergistic, antagonistic, or can potentiate the response expected from individual component exposures. This is the complex problem that faces environmental scientists and public health officials in setting and carrying out public health policy for the general environment, consumer product and food and drug safety, and the protection of workers. Because the issue of mixed exposures affects all of these areas, it was selected as one of the priority areas of the National Occupational Research Agenda (NORA) to leverage collaborative research efforts for better understanding the complex interactions of mixed exposures.

The mixed exposures research agenda includes the elements generally found in public health responses: surveillance, evaluation and research, and controls and interventions. Health surveillance is needed to identify mixtures with adverse health effects that cannot be explained by the toxicity of the individual components in a mixed exposure. Exposure surveillance is needed to identify workers exposed to mixtures with observed potential effects. To create manageable priorities for research and worksite interventions, systems are needed for ranking mixed exposures on the basis of knowledge about health effects and the degree to which exposure is likely to occur.

In addition, the research agenda describes a variety of evaluation tools that can be used to assess the risk of exposure to various mixtures. Additional research is needed to develop better tools for toxicity analysis, exposure-response modeling, and physiologically based pharmacokinetic and pharmacodynamic (PB/PK and PB/PD) modeling. An approach based on observed health effects and observed exposures is needed to control exposures to mixtures and to assure that protective technologies are not compromised by multiple simultaneous exposures. For example, the service life of respirator cartridges may be reduced by the presence of an interfering agent. Finally, the research agenda identifies intervention opportunities and information dissemination needs to assure that the outcomes of the developed research can be applied to preventing harmful effects of mixed exposures.

Because resources are limited, the Mixed Exposures Team identified several research needs as top priorities. They are listed below:

- Develop and implement new surveillance methods to identify the number of workers exposed to these mixtures, the range of exposure concentrations, and health effects associated with the mixed exposures.
- Develop research strategies that promote collaboration between occupational health professionals and workers in ranking and characterizing mixed exposures within specific occupations and industries. Such assessment will also facilitate dissemination of research findings.
- Conduct research to better understand the toxicology (biological mechanisms) of mixed exposures.
- Develop methods to understand and integrate experimental data from the molecular level to the whole organism. For example, researchers should develop the ability to use data from proteomics* and genomics† studies and extrapolate these to whole body systems.
- Develop methods that can be used to measure and predict deviations from additivity.
- Develop and validate mechanism-based exposure-response models.
- Develop the concept of the *virtual human* by means of PB/PK simulation.
- Develop default parameters for mechanistically based risk estimation and extrapolation models.
- Develop biosensors or measurement technologies (such as micro-arrays with advanced signal processing) that indicate whole mixture toxicity.
- Identify, validate, and characterize the health outcome for biomarkers of exposure and response for workers exposed to mixtures.
- Determine the effects of mixtures on engineering controls and personal protective equipment (PPE); evaluating each mixture's potential to adversely affect the protection provided by the controls.

Through these research advances, policymakers and regulators may be better able to assess the true risk involved in most occupational and environmental exposures that include multiple stressors and mixed-chemical exposures.

*Proteomics: The study and analysis of protein structure and function.

†Genomics: The study of the structure and function of large numbers of genes observed simultaneously.

Abbreviations Ê

ACGIH	American Conference of Governmental Industrial Hygienists
AIHA	American Industrial Hygiene Association
ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CNS	central nervous system
CPWR	Center to Protect Workers' Rights
DoD	U.S. Department of Defense
DNA	deoxyribonucleic acid
DOE	U.S. Department of Energy
EPA	U.S. Environmental Protection Agency
GC-MS	gas chromatography-mass spectrometry
HI	hazard index
LD_{50}	lethal dose of a compound for 50% of the animals exposed
MAK	Maximum Workplace Concentration (Maximale Arbeitsplatz Konzentration)
MSDSs	material safety data sheets
MSHA	Mine Safety and Health Administration
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NORA	National Occupational Research Agenda
OSHA	Occupational Safety and Health Administration
PAHs	polycyclic aromatic hydrocarbons
PB/PD	physiologically based pharmacodynamic

PB/PK	physiologically based pharmacokinetic
PPE	personal protective equipment
psi	pounds per square inch
QSAR	qualitative or quantitative structure-activity relationship
RfC	reference concentration
RfD	reference dose
SARs	structure-activity relationships
STEL	short-term exposure limit
TAFE	Technical and Further Education (New South Wales)
TEF	toxicity equivalence factor
TLV	threshold limit value
TNO	Toegepast-Natuurwetenschappelijk Onderzoek (Netherlands Organization for Applied Scientific Research)
TTC	target organ toxicity concentration
TTD	target organ toxicity dose
WOE	weight of evidence
WPAFB	Wright Patterson Air Force Base

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1 Introduction Ê

Background

The importance of mixed-exposure research for controlling the occupational environment and the decision to address this issue are driven by the following factors: (1) the concern for the known and perceived health risks from mixed exposures in the workplace, (2) the existing regulatory mandates, and (3) the current state of science.

Two examples of known or perceived health risks with toxicological endpoints and consequences from mixed exposures are (1) loss of hearing because of noise and chemical interaction and (2) synergistic carcinogenesis of asbestos and smoking. Other examples show more uncertainty. The Gulf War syndrome and the mixed-exposure-associated health effects from jet fuel (JP-8) exposures are far from clear. This lack of clarity stems from the complex nature of the mixtures involved and their related biological consequences. These mixtures not only interact within the human system, they can also undergo chemical transformations in the environment. Examples of this transformation are the conversion of some chlorinated hydrocarbons into toxic phosgene in the presence of ultraviolet light and the enhanced transport of radionuclides into the lungs when adsorbed by respirable dust.

Regulatory mandates in 29 CFR* 1910.1000 provide exposure limits for air contaminants in general industry. This regulation specifies an *exposure additivity formula* to compute reduced workplace exposure limits for chemical mixtures. Lacking, however, are detailed guidelines to help occupational hygienists apply the additivity formula, determine its appropriateness for different situations, and identify the degree to which sufficient protection is provided. For example, little information is available to guide occupational hygienists on when to apply the exposure additivity formula, when to consider the effects of multiple exposures as *independent*, and when *synergistic* or *antagonistic* effects may be expected.

The U.S. labor force continues to grow both as a percentage of the overall population and in total number. In 1998, 138 million people (67% of the population) made up the American workforce. The workforce is expected to grow to 158 million in 2010 [Fullerton and Toossi 2001]. The fundamental mission of the National Occupational Research Agenda (NORA) is to shape research aimed at delivering on the NIOSH vision to provide ". . . safety and health at work for all people." With more than 5,900 workers killed on the job in 2001 and far more dying prematurely of occupational disease, we are short of realizing our national goal of a *safe and healthful* workplace for all [BLS 2002].

Over the past 20 years, the topic of mixture research has witnessed a transition from outright avoidance to carrying out simple, descriptive studies of binary mixtures to planning and carrying out sophisticated studies using new technologies in biological and computational

Code of Federal Regulations. See CFR in references.

sciences. The complete elucidation of the human genome, the related developments in genomics and proteomics, and the exponential growth of computational technologies provide essential opportunities to deal with the effects of mixture exposures on complex biological systems. The current state of science is right for addressing research in the complex area of multiple stressors.

National Occupational Research Agenda (NORA)

One of the principal goals for the NORA initiative is to develop research priority areas leading to the protection of the workers' health. Since occupational exposures to chemical mixtures and multiple stressors is the rule rather than the exception, we are committed to finding ways to tackle the complex area of health effects of such mixed exposures.

The impetus behind the NORA Mixed Exposures Research Agenda is the need to answer three fundamental questions:

- How can we detect mixed exposure effects and gain information sufficient for scientifically based decision making?
- How can we predict mixed exposure effects?
- Which intervention or avoidance strategies will be most effective for mixed exposures?

In fact, all exposures are *mixed exposures* in the sense that none occur in isolation from exposures to other simultaneous or sequential stressors inside or outside the workplace. Because NORA cannot plausibly address the full universe of possible mixed exposures, the scope of research targets must be limited.

Thus the identification and prioritization of key exposures is a very important step in developing a research agenda and protecting the health of workers.

Identifying Mixed Exposures

Mixtures of concern may be identified by the following three criteria:

- A large number of workers are exposed to the mixture.
- The health outcomes of exposure to these mixtures are of a nature or magnitude that cannot be explained with our current knowledge of single exposures.
- Exposures to these mixtures have health outcomes predicted from known effects of individual exposures that are also known to occur together.

For the first criterion, Table 1 illustrates the magnitude of worker exposure to some of the most widely encountered workplace mixtures.

For the second criterion, identification is driven by health problems. For example, a strong interaction of asbestos exposure with cigarette smoking was recognized after increased lung cancer rates were observed among asbestos workers.

For the third criterion, identification is driven by the knowledge of individual components' physiological effects. An example of this is exposure to methylene chloride with co-exposure to carbon monoxide. Both agents reduce the blood's capacity to carry oxygen by formation of carboxyhemoglobin, thereby potentially increasing the risk of cardiovascular health effects.

Table 1. Common mixed exposures for workers. Ê

Exposure agent(s)	Disease or outcome (known or suspected)	Estimated number of workers exposed
Fuels and combustion products	Cancer, chronic obstructive pulmonary disease, pulmonary function changes, chemical pneumonia, central nervous system (CNS) effects, liver or kidney damage, irritation of eyes, skin, or mucous membranes	> 10,000,000
Chemicals and noise	Hearing loss	4,700,000
Welding fume	Cancer, respiratory disease, metal fume fever, eye damage, neurological impairment	760,000
Asphalt fumes	Irritation, chronic obstructive pulmonary disease, cancer	470,000
Chemicals and radiation	Cancer, immune dysfunction, eye and skin damage, CNS effects (from the chemical exposures)	400,000
Metalworking fluids	Contact or irritating dermatitis; hypersensitivity pneumonitis (that is, hypersensitive pulmonary alveolitis); suspected to cause cancers of some organs	340,000

Sources: National Occupational Exposure Survey and National Occupational Health Survey of Mining [NIOSH 1990, 1991].

Any chemical, physical, or biological insult on the body is a form of stress; therefore, multiple stressors can include chemicals, drugs, and physical and biological agents [Yang 2000]. However, the domain of multiple stressors may be much wider and certainly should include psychological stress. Although some of these stressors may have been studied individually and reported in the literature, little or no information is available about the possible combined actions of multiple stressors. Stress is defined as a state of disharmony or threatened homeostasis [Chrousos and Gold 1992]. If the homeostasis is disrupted because of physical or psychological stress including social and socioeconomic stress, intricate neural and biochemical events in the brain and in the endocrine and immune systems act jointly to counter the effects of stress and to reestablish homeostasis [Ember 1998]. If homeostasis is not reset, debilitating illness can result.

Identifying mixed exposure hazards requires a two-tiered approach. First, a better understanding of work processes and materials is needed. Ideally, such information will be collected as close to the workplace as possible. The second tier of research (on which much

of this report is based) involves development of stronger scientific methods with which to measure potential health effects resulting from mixed exposures. Mixed-exposure research will require the development and refinement of mathematical and physiological models that can be used to estimate the effects of stressors on whole body systems. To be successful, substantial improvements are needed in our knowledge of biological mechanisms of toxicity, chemical structure-function relationships, and dose-response relationships. Such knowledge would in turn lead to the development of biological screening tools and improve our ability to model exposure-effect relationships. Advances in this direction should be fostered, at least initially, by focusing mixed model research on systems that have already been defined.

Toxicological research and the complex science needed to measure the combined heath effects of multiple stressors on the human body are limited by the quality of exposure data. In other words, our understanding of potential health effects resulting from mixed exposures is only as good as our understanding of what workers are exposed to throughout their working lives. The fact that both mixed exposures and exposure assessment methods were among the top 21 research priorities for NORA illustrates the fundamental and interrelated importance of finding better ways to understand real-world exposure patterns in the modern work environment and to determine associated health risks.

Scope

This document sets forth a research agenda for occupational exposure to mixtures that should serve as a blueprint for building a national research program. By identifying high-priority research areas, this agenda should influence the allocation of research resources. Developing a public health approach for preventing disease and injury resulting from mixed exposures is a daunting task. Workers are commonly exposed to multiple agents—as mixtures of agents, as separate simultaneous exposures, or as sequential exposures. Mixed exposures present a seemingly intractable problem for health professionals dealing with occupational safety and health issues, environmental health issues, and food and pharmaceuticals to name a few. The present substance-by-substance or stressor-by-stressor approach to hazard control is inadequate. The true risk to workers is likely to be underestimated when considering each stressor independently. This document identifies priority research needs for occupational safety and health. For the various agencies and stakeholders with an interest in mixed exposures, this research agenda clarifies areas of mutual interest, discusses new technologies and research tools, and improves our common understanding for dealing with mixed exposures.

For the purposes of this document, mixed exposures include chemical mixtures as well as mixed stressors such as exposure to physical agents (for example, noise, heat, radiation, and vibration) or other physiological stresses associated with work (for example, psychological stress). Chemical mixtures may be intrinsically complex mixtures (diesel exhaust, fuels) or identifiable component mixtures (benzene-toluene-xylene) that are sometimes called *simple mixtures*. Exposure to these agents may occur simultaneously or sequentially, producing cumulative risks for workers. Outside the workplace, workers will be exposed to other agents (such as pharmaceuticals, food additives, alcohol, or tobacco smoke) that may interact with various workplace chemical and physical agents, potentially creating new and unhealthful or

unsafe conditions. This document will not address food and pharmaceuticals explicitly, although the importance of these exposures is recognized. In addition, issues related to individual susceptibility (which a separate NORA Team addresses) will not be the focus of this document.

The research agenda described in the following sections reviews various approaches that have been taken to address the problem of mixed exposures including hazard identfication, effects, exposure and risk assessment, and control.

With each approach, the team has identified knowledge gaps and opportunities for intervention. Finally, in recognition that resources are always limited, the final section of the research agenda seeks to identify principles for prioritizing mixed-exposure research as well as a few high-priority activities that, if completed, would provide the greatest leverage in offering useful public health guidance.

2 Analysis of Research Needs Ê

Identifying Mixture Hazards

Identifying and characterizing mixed-exposure hazards have always been challenging. However, as we continue to shift away from a manufacturing-based economy, the challenge will be even greater. The National Academy of Science report entitled *Safe Work in the 21st Century—Education and Training Needs for the Next Decade's Occupational Safety and Health Personnel* [Institute of Medicine 2000] identifies several trends and projections related to the U.S. workforce:

- The proportion of workers over age 55 will increase in the next decade, as will the number of workers in the youngest group (aged 16 to 24).
- Workers are likely to hold more jobs and will change jobs more often than in past generations; work is more likely to be contracted out than in past years.
- With the exception of construction, the goods-producing sector of the economy (mining and manufacturing) shrank in the 1990s.
- Millions of service-sector jobs—including health care—were added to the economy during the same period.

Given these trends, chronic occupational disease is likely to be of growing importance as the age span of the workforce increases. We know from existing surveillance data that exposures encountered at an early age can have life-threatening consequences well after exposures have ceased. The death of two sandblasters from silicosis in 1998, each with less than 5 years of employment as sandblasters, serves as a tragic illustration of this fact [CDC 1998]. One of the deceased workers was employed as a sandblaster in his twenties between 1984 and 1988. He died at age 36. The second worker was only 30 when he died. He had started working as a sandblaster at about the age of 18 between 1986 and 1990. Fortunately, most young workers will live long enough to pursue a variety of occupations. However, the lifetime exposure profiles of such workers will be increasingly complex as younger workers cycle into and out of different jobs and the job materials and processes change. Without better control of health hazards, the risk of chronic disease will also increase as workers enter the workforce at an earlier age and leave at a later age.

To get a clear view of exposure potential within the workforce and prioritize research areas, we must draw on the knowledge of those doing the work. Occupational hygienists, epidemiologists, toxicologists, and occupational health professionals will continue to be of critical importance in the development of exposure assessment strategies and analysis of data. However, understanding how work is done, what materials are used, and which mixed-exposure hazards pose the greatest concern in the workplace make up the foundation on which all other research rests. Therefore, central in our research agenda should be the

development and use of research methods and partnerships that involve workers more actively in the research process. Two methods that have been successfully used toward this end are participatory research methods and research that involves the training and use of workers as *shop-floor* gatherers of both qualitative and quantitative exposure data.

Participatory research involves a co-learning process in which research subjects and professional researchers become active partners in the process of identifying occupational health problems and interventions that are likely to take hold within the group being studied. The general approach involves more emphasis on developing a system for addressing problems as they arise in a real-world context [Rosecrance and Cook 2000; Schurman 1996]. Such methods hold great promise in tackling the very complex and idiosyncratic problem of mixed exposures.

The practicing occupational hygienist in the field has long known the value of talking to workers to understand a given work process. In addition, the Occupational Safety and Health Administration (OSHA) and Mine Safety and Health Administration (MSHA) standards such as the Hazard Communication Standard or other agent-specific standards and well-known diseases such as asbestosis have increased worker awareness and interest in occupational health. However, the complexity of process materials and problems with hazard communication tools such as material safety data sheets (MSDSs) limit worker knowledge of mixed-exposure hazards. A study carried out by Worksafe Australia and the New South Wales Technical and Further Education (TAFE) involving apprentice painters describes the limitations of MSDSs in the context of solvent thinners [Winder and Ng 1995]. In their review of MSDSs for 20 paint thinner products, 83 chemical solvent ingredient names were listed as hazardous ingredients. These 83 ingredients were reported as present in wide ranges (as opposed to a percentage of the by-product formulation), making precise product formulation unclear. In addition, the 83 trade-specific or generic thinner components could be reduced to 32 solvents or 6 classes of solvents. The authors emphasize the importance of product formulation in characterizing exposure risk. They also conclude that information about mixed-solvent exposure is lacking and that educational programs are needed to help workers understand various chemical formulation risks.

Although the above research focused on paint thinners, the painting trades in general offer a rich illustration of the complexity of mixed-exposure risk. Industrial painters, for example, routinely begin their jobs by blasting steel surfaces covered with coatings (usually lead-based). Abrasives such as silica sand, steel grit, and copper or coal slag are used to blast old paints off steel and concrete surfaces using high pressures (about 100 pounds per square inch [psi]). The dust generated as a result will consist of an array of hazardous agents (including fibrogenic dusts or metals), depending on the abrasive and substrate. The individual metals and dusts can cause a wide range of serious health effects; however, the combined effects of these agents have not been studied. Paint systems that are applied to the freshly prepared surface produce paint mists and solvent vapors that are even more complex mixtures of hazards.

The construction industry serves as a good model for mixed exposures. In this industry, both old and new sources of exposure pose a threat to a wide range of trades and occupations. For example, pipe fitters may be exposed to the fumes of new high-nickel alloy welding rods while working on construction of new semiconductor facilities, and on another job they may be exposed to asbestos applied to process piping a generation ago.

Masonry workers repairing old mortar joints are exposed to dust containing silica as well as to hazardous materials mixed into the mortar decades ago. Regardless of the source of mixed-exposure hazards, they translate into the potential cause for future occupational disease.

The mining industry also serves as an excellent model for mixed exposures. In this industry, miners may be exposed to the particulate matter released from diesel engines that is combined with an irritant gas such as nitrogen dioxide and an asphyxiant such as carbon monoxide. Miners may also be exposed to mixtures of solvents in cleaners or to metals and thermal degradation products generated during welding. And like in other industries, these mixed exposures translate into a potential cause for both present and future occupational disease.

The historical *one-chemical-at-a-time* approach to occupational health is inadequate. Safety and health practitioners using substance-by-substance or hazard-by-hazard approaches generally make conclusions about worker risk or lack of risk without sufficient caveats about the inability to evaluate additive or synergistic effects. However, the problem of understanding the true health effects of *real-world* mixed exposures is mind-boggling unless systems are in place for clarifying research priorities within major occupational groups. Workers and organizations that represent or train them are essential building blocks for developing such systems. To accurately assess mixed-exposure potential, professional researchers must collaborate with workers, the organizations that train and represent them, and entities that influence how work is done. Collaboration between scientists, engineers, management, workers, and others is needed for identifying and ranking exposure hazards. Use of such approaches has been described in the literature [Anderson-Murawski et al. 2002; Feron et al. 1995b].

Screening methods may be used to systematically evaluate multiple exposures. First, known occupational exposures are ranked by frequency of occurrence. Then combinations are ranked by the frequency with which two or more exposures occur together. Finally, the resulting list of combinations is reviewed to identify those for which present knowledge suggests that interactions may occur.

A key criterion for identifying mixed-exposure research priorities is the anticipated outcome. Ideally, the NORA Mixed Exposures initiative would result in the evolution of a much improved ability to predict important potential mixed-exposure threats in materials before they enter into commerce. Of course, this presumes that new combinations of such stressors would be known and reviewed in advance, and that employers would act on such knowledge. The likelihood of this scenario depends on the degree to which workers, occupational safety and health professionals, and those in industry responsible for selecting work process and materials are involved in the hazard prioritization process.

Another gap is the need to provide information about common mechanisms of toxicity. The American Conference of Governmental Industrial Hygienists (ACGIH) specified that for chemical mixtures, one should total the exposure-dose contributions from multiple agents that *affect the same organ system.* Only recently (1998) has the ACGIH published the *Critical Effects* associated with each chemical in their threshold limit value (TLV®) booklet [ACGIH 2003]. However, when there are more than one critical effects listed, it is unclear on which effect(s) the TLV was based. In addition, the mechanism of action is not specified in the TLV book. Thus, the documentation for each TLV must be consulted

for this information. Likewise, the Food Quality Protection Act of 1996 (Public Law 104-170) requires the U.S. Environmental Protection Agency (EPA) to consider all non-occupational sources of exposure, including drinking water and exposure to other pesticides with a *common mechanism of toxicity* when setting tolerances. These approaches create a need for additional knowledge about toxicity mechanisms in addition to which organ system might be affected.

New Knowledge Needs

- Develop a greater understanding of historical and current mixed exposures by occupation within targeted industries, including underserved and growing sectors such as construction.
- Develop research strategies that promote collaboration between occupational health professionals and workers in ranking and characterizing mixed exposures within specific occupations and industries.
- Develop and implement new surveillance methods to determine the number of workers exposed to various mixtures, identify the range of exposure concentrations, and identify health effects associated with mixed exposures.
- Develop information about occupational hygiene practice to determine (1) the extent to which occupational hygienists, compliance officers, medical personnel, and other occupational safety and health professionals use mixed-exposure information, and (2) whether they make any adjustments for the combined effect; either as additive or synergistic effects.
- Develop information about common mechanisms of toxicity. A database of known mixtures and their mode-of-action could assist occupational hygienists.
- Conduct research on *in-place* mixed-exposure hazards as encountered in the mining, construction, service, and manufacturing sectors.
- Conduct research on new materials that introduce new mixed-exposure hazards into the workplace; for example, high-nickel alloy welding rods, concrete additives, and slag and mineral abrasive material.
- Conduct surveillance research to describe the severity of the effects from mixed exposures.
- Gain a better understanding of the ability of nonchemical stressors to affect an exposed person's tolerance of exposure to chemicals.
- Gain a better understanding of the ability of chemicals to affect an exposed person's tolerance of physical agents to such as noise.

Potential for Intervention

- Once mixed-exposure issues are identified, research priorities can be set on the basis of factors such as (1) the frequency with which the exposures occur and the number of workers affected, (2) the severity of the effects, and (3) the likelihood that research will effectively prevent occupational disease.
- New information will lead to the development of information tools (for example, more informative and ac-

curate MSDSs), education and work-based systems for recognizing and characterizing mixed exposures, improved analytical methods, better sampling methods, and more comprehensive and systematic approaches to collecting exposure data.

- Partnerships for intervention will be developed and will include unions, apprenticeship and training programs, materials engineers, and industry organizations. These partnerships will identify, create, and market engineering controls for reducing mixed-exposure risks.

- Control technology research will be aimed at broader hazard focus areas—for example, dust-control systems that eliminate multiple hazards or substitute materials that do not introduce new hazards.

- Existing mechanisms can be more effectively used for characterizing and controlling mixed exposures. These mechanisms include joint labor/management committees and process hazard analysis systems, which are required in a number of industries under the OSHA Process Safety Management Standard.

Effects Studies

Experimental and epidemiological research on mixed exposures addresses two interrelated issues:

- Understanding, predicting, and screening effects of specific mixed exposures

- Predicting and screening key effects across a broad range of exposures

Knowledge gained in both areas is used to improve risk assessment and mitigation measures. Many research tools and strategies address both issues. For example, recent advances in mechanistic models and cellular and molecular research tools (such as genomics, proteomics, and bioinformatics) have enhanced our ability to address questions about possible health effects in the areas of both specific and generalized mixed exposures.

Other methods and tools focus more on one type of mixed-exposure issue. For example, research targeted for specific mixed exposures commonly employs basic toxicological strategies such as cellular, animal, and human studies to focus on exposure patterns, metabolism, response mechanisms, biomarkers, susceptibility, and health outcomes. Such research typically relies on exposure-dose-response relationships to identify and characterize interactions. It tends to be retrospective, typically addressing existing or historic exposures. But such research can also be applied to anticipated or hypothesized exposures. On the other hand, research targeted for generalized application typically focuses on developing more rapid, effective, and inexpensive ways to predict interactions across a variety of exposures, and it often involves cellular models, physiologically based pharmacokinetic (PB/PK) and physiologically based pharmacodynamic (PB/PD) models, chemical structure-activity relationships (SARs), and mathematical tools and data analyses for generalizing relationships across classes of stressors and exposures that lead to different classes of effects. Such research tends to be prospective, although known interactions may serve as a starting point. In either case, it is important to consider multiple effects beyond the critical (most sensitive) effect to evaluate possible combined responses to multiple stressors and exposures.

Experimental Approaches

Mixed exposures are too complex and variable to prescribe any single approach as *most appropriate* for understanding related health effects. Research in this area must offer a way to focus on key health issues considering an overwhelming number of permutations of types and sequences of exposure to multiple stressors in the workplace, as well as interactions among workplace and nonworkplace exposures. The fundamental methods and tools used for this research build on those used for other basic and applied research, and improvements will certainly continue in concert with advances in those research fields. However, the uniqueness of mixed exposures warrants targeted research to understand and predict combined human responses. This research strategy will involve both adaptations to existing approaches and the development of new methods and tools for characterizing joint health effects and their key contributors and modifiers.

Experimental and epidemiological research opportunities span a wide range of biological levels and study species—from molecular, cellular, and tissue studies to whole-animal studies, and from microorganisms and standard test animals to human subpopulations and populations. These studies, together with mathematical and visualization approaches, can be used to evaluate and predict possible human responses to multiple stressors and exposures. For example, especially useful for mixed-exposure research are physiologically based mathematical and statistical approaches such as PK/PD models. These represent integrated exposure-dose-response relationships including response surfaces and integration of approaches to group classes of interactions, toxicity endpoints, stressors, and exposure types.

Laboratory Research

Cell Models

Cell culture systems using either established cell lines or primary cultures are attractive because of their simplicity and low cost. A wide range of cellular response phenomena can be observed through assays of cell function, general cytotoxicity (that is, survival, multiplication, surface adhesion, confluency, etc.), phenotypical changes, gene expression, and protein production. Cell models are also useful for studying interactive mechanisms such as P450 interactions in PB/PK models [Olin 2004]. They may also be useful as bioassays, assessing whole mixtures. The teaming of cell models with rapidly developing molecular biology investigative tools is expected to play a significant role in research on mixed exposures. Large-scale studies on the nature of chemical interactions, studies aimed at lumping responses among chemical classes, and high-volume prospective screening of chemicals for interactions will probably rely more on using cultured cells than on intact animals.

Cell models have limitations as well as advantages. Immortalized cell lines are necessarily altered from their source cells. Primary cultures have limited life spans. No culture technique fully mimics the *in vivo* environment. The value of information derived from cells in culture corresponds directly to the level of confidence that *in vitro* responses reflect the *in vivo* responses and dose-response relationships. Although intracellular phenomena can be identical in the two settings, responses that are influenced by other cells *in vivo* may not be reflected well in cultures of a single cell type. This issue places a premium on validating cell responses against *in vivo* responses to avoid generating large databases on mixed exposures of uncertain applicability to humans.

Animal Models

The use of intact animals allows adverse effects and interactions among exposures to be evaluated in the presence of the integrated responses from all organ and tissue systems. Although responses occur in single cells, adverse health effects in intact persons seldom, if ever, occur without the participation of multiple cells, tissues, and organs in pathogenic, defensive, or reparative responses. Animals can be studied using nearly the full range of morphological, physiological, and biochemical assays applied to humans. In many cases, large databases are available for comparison, although caution must be exercised to consider differences among the animal strains and experimental designs used in different studies. Intact animals are probably the only model adequate for evaluating mixed stressors (other than chemicals), such as physical stressors (for example, extreme cold or heat, exercise, radiation), personal factors (for example, nutritional deficiencies, aging, etc.), hormonal changes (for example, menstrual cycles, pregnancy), biological stressors (for example, infectious agents), and psychological stressors. Intact animals are also required to study reproductive (for example, fertility, teratological) and postnatal development and growth phenomena, although the latter is seldom a workplace issue.

Statistical Tools

To address mixed-exposure issues, the research tools must be deployed in experimental designs tailored to the question being asked or the hypothesis being tested. The large number and complexity of potentially important mixed exposures place great premium on the design of fundamental research strategies aimed at understanding and predicting the effects of combined exposures. No single research strategy will meet the need. A number of different strategies have been used in the past [Mauderly 1993], and continued development in this field is needed. This section briefly describes common fundamental strategies for mixed-exposure research. These examples are illustrative but not exhaustive. All of these strategies have both strengths and limitations, and any of them could be the best, depending on the question being asked and the resources at hand. In general, the same fundamental strategies could be applied whether the issue is combinations of dissimilar (for example, physical and chemical) or similar exposures (for example, complex chemical mixtures in a single exposure medium). It is important to note that continued development of research strategies is needed, as well as development of research tools.

Research Organized Around Prioritized Lists of Exposures

Another strategy is to prioritize a larger but still limited list of exposures and use a range of experimental protocols to verify the assumptions of additivity or independence of effects of the exposure combinations [Feron 1995b]. This is primarily a prioritization strategy; most, if not all, of the other strategies described in this section could be applied to the list of exposures. This strategy is useful for focusing research on key components of highly complex mixtures. However, this strategy denies the complexity of the *real* exposures, relies on foreknowledge of the most important components, and faces overwhelming, even if limited, permutations.

Studies of Sequences of Exposures

Mixed-exposure issues include sequences of multiple exposures as well as multiple simultaneous exposures. Several experimental designs can be used to study sequences, but the unifying feature is the administration of exposures at different times [Mauderly 1993]. The experiments may involve simply reversing the order of two exposures, or they may incor-

porate a factorial design in which single exposures, simultaneous combined exposures, and the two (or more) sequential exposures are administered. Like any factorial design, sequential experiments become intractable when the number and thus the possible sequences of exposures increase.

Dissection of Effects of Complex Exposures

This strategy focuses on apportioning causation among multiple (typically many) components of mixed exposures [Mauderly 1993]. It begins with a known effect and a known or assumed combination of exposures, and it attempts to identify the causal factors. This strategy has proved useful in many cases—and especially for mixtures for which the mechanism of action is similar (for example, mutations). However, the strategy depends on the ability to reproduce the exposure (that is, the mixture) and its isolated components. This strategy can also be resource-intensive if the number of components is large, the separation difficult, or the biological test system complex. Experimental designs that aim to determine the components of a mixture responsible for an effect are often termed *bio-directed fractionation* [Schuetzle and Lewtas 1986; Kleinman et al. 2000; Rudell et al. 1999].

Multivariate Analysis of Variable-Exposure Versus Response Databases

This strategy focuses on statistical analysis of matrices of exposure-response data in which identical measures of response are applied to multiple mixed exposures that differ in composition. The approach takes advantage of differences among the exposures (for example, in the composition of the exposure material) to identify the components most strongly associated with the effect(s). In a sense, this strategy is similar to bio-directed fractionation, except that it depends on variations in the complex exposure rather than on dissection of a constant complex exposure. The biological response system can range from simple (for example, single mutation in cultured cells) or complex (for example, multiple health outcomes in animals or humans). This strategy can address complex exposures and can use either epidemiological or laboratory data. However, its success depends on the consistency of populations and response measures across exposures, the accuracy with which individual exposures are known, and the degree of detail and similarity with which the different exposures are characterized [Eide et al. 2002; McDonald et al., in press].

Epidemiology

In research on mixed exposures, appropriate roles will exist for studies involving humans or data collected from humans, as is the case for research on single occupational and environmental exposures. Valuable data on subclinical responses can be obtained from humans exposed experimentally (that is, clinical studies) to concentrations and combinations of physical and chemical exposures considered to be without significant risk—with appropriate precautions and involvement of institutional review boards. Perhaps the most common human research is epidemiology, in which adverse health outcomes are linked to exposures retrospectively or prospectively.

All epidemiological studies deal in mixed exposures, along with the other contributing factors. The challenge to our research methods is to identify the exposures that contribute to the disease process and distinguish them from exposures that do not contribute. This is particularly difficult for epidemiological studies with mixed exposures because detailed exposure data is usually lacking, the relative concentrations of the mixture components will be variable over time, and the ef-

fects often involve chronic disease endpoints with long latency periods.

Epidemiological studies can be broadly categorized by purpose as descriptive or analytical. Descriptive studies involve observation of demographic, secular, or geographic trends in the occurrence of health outcomes. Analytical studies attempt to determine the relationship between outcomes and exposures or other risk factors and require clear specification of the outcomes and risk factors of interest. These risk factors include individual occupational exposures but more frequently, combinations that constitute mixed exposures. Epidemiological studies are generally either cohort or case-control, distinguished by whether the populations are first defined by exposure (cohort) or by health outcome (case-control). Cross-sectional and prospective studies can be especially suitable for examining mixed exposures. By design in a cross-sectional study, exposure and outcome measures are assessed simultaneously. Direct access to the study subjects and to the environments in which they may be exposed to potential causal factors allows detailed assessment of the components of mixed exposures.

Modeling Approaches

In mixed-exposure research, it is important to obtain quantitative information about the time-course fate and locations of chemicals and metabolites in the body (that is, PK) and time-course of receptor interactions and toxic responses (that is, PD). Such information is important for understanding the mechanistic basis for interactions among chemicals and thus predicting interactions and extrapolating from cell and animal studies to humans. Mixed-exposure research must include studies integrating computational technology and mathematical/statistical modeling with mechanistically based, time-course toxicology studies. This field is rapidly evolving and lends itself well to taking strong advantage of recent advances in cellular/molecular biology, mathematical models of biological responses, and mathematical lumping strategies for aggregating responses to classes of chemicals. The potential for significant advances in this field have been described by Yang et al. [1998].

A principal aim of dose-response modeling is to develop predictive tools for health risk assessment—that is, to be able to extrapolate likely biological effects observed in experimental situations to realistic human exposure situations (for example, to low doses, different species, routes of exposure, etc.). Such extrapolation is possible only with a quantitative understanding of the underlying mechanisms of absorption, distribution, metabolism, and elimination. The importance of understanding mechanisms for the effects of mixtures is twofold:

- To understand behavior of individual components (as a baseline)
- To understand interactions between components

Over recent years, perhaps the most important development in this area has been the development of methodologies for PB/PK modeling of the chemical behavior in the body that takes into account the underlying physiology of the species of concern.

Physiologically Based Pharmacokinetics (PB/PKs)

PB/PK modeling is an approach that attempts to predict biological effects from the perspective of the entire biological system; it allows for development of a biologically accurate toxicokinetic description of an experimental mode that incorporates flow and dose relationships, realistic tissue volumes, solubility

parameters for individual species and chemicals, and metabolic pathways with measured kinetic parameters. PB/PK models take the known pharmacodynamics of the chemicals (identified through *in vitro* studies or studies in other species) and information about possible interactions and use that known information to predict the overall toxic effect level of any dose or ratio of the mixture. These models take into account all the processes of a cell that could influence toxicity, including transport processes, diffusion exchanges, metabolic and eliminatory clearances, and receptor binding.

This technique can be used to extrapolate data between chemicals or to generate predicted chemical interactions that may be tested in the laboratory. This approach can be more resource efficient than traditional testing for multiple possible mixtures, many of which may prove not to have had any relevant interactions [Bond and Medinsky 1995]. Chemical-specific factors such as blood-tissue and tissue-tissue partition coefficients, elimination rate constants, and metabolic rate constants are determined *in vitro* and then used to create the predictive model.

PB/PK models can be adapted to make toxicokinetic predictions for specific organisms or target organs, and the model can be used in some circumstances to predict the concentrations necessary for a toxic effect. PB/PK modeling is also useful in making flow and dose predictions. Although modeling does not replace well-planned laboratory experiments, it is a useful tool that can facilitate experiment planning, optimize the use of laboratory data, and help design cost-effective studies [Blancato 1994].

PB/PK models have been developed for several components of the jet fuel JP–8 (such as benzene, xylene, toluene, and nonane). In addition, models of the interactions of up to five component mixtures of chemicals have been studied [Haddad et al. 1999; Tardif et al. 1997]. A key result of these studies is that a complete description of the interactive processes can be obtained by simultaneously tracking all the binary interactions in the mixture (that is, interactions of one chemical with another). Higher-order interactions are automatically taken into account in this way. Analysis of the blood kinetic data suggested that competitive metabolic inhibition of P450 2E1 was the most likely interaction mechanism for these compounds, and the metabolic inhibition constants for each binary interaction were determined. These results can be generalized to an arbitrary number of similarly acting mixture components (such as the hydrocarbon components of fuels) by considering such complex mixtures as pseudo-binary systems consisting of the compound of interest plus a single interacting complex *vehicle* with well-defined composite properties. Such composite properties (such as inhibition constants in the present example) are model-based statistical averages of the values for each interacting component. Such pseudobinary systems could be investigated by modifying the techniques developed for true binary interactions such as response surface analysis (see *Mathematical Modeling Tools* below).

Qualitative or Quantitative Structure-Activity Relationships (QSARs)

Approaches using QSARs attempt to predict the effects of a chemical mixture by making analogies with other similar compounds. They are useful for chemical mixtures for which limited dose-response data are available. SARs identify a common substructure or similarity in form among compounds with similar modes of toxic action; they then use the presence of this substructure in another mixture of unknown toxicity to predict toxicity in that compound. QSARs attempt to define quantitative structure parameters that

correlate with an experimental concentration that produces the identical effect, such as an LD_{50} (the lethal dose of a compound for 50% of the animals exposed). QSAR techniques are used to predict vital chemical parameters for unknown compounds such as partition coefficients, metabolic rate constants, and elimination constants as well as possible pharmacodynamic parameters such as binding affinities and maximum turnover velocities for target enzyme systems. QSAR techniques can also help to determine parameter values for PB/PK models (for example, partition coefficients and penetration coefficients), especially those to which the model output is not particularly sensitive. Thus the need for experimental determinations is considerably reduced. The QSAR techniques are limited by the availability of underlying structure-based data used by the models.

Lumping Analysis

The technique known as lumping analysis is borrowed from the petroleum industry, in which chemicals with defined similarities are lumped together into pseudo components that represent the entire group to make analysis scientifically manageable. This technique allows modeling approaches on very large mixtures (20 or more components). The sheer numbers of mathematical manipulations required for such mixtures would otherwise prove overwhelming to most computer systems. The complexity of the analysis is reduced by treating compounds with similar structures or mechanisms of actions as one chemical.

A new approach called structure-oriented lumping has been developed to model the composition and chemistry of complex mixtures at a molecular level. The central concept is to represent an individual molecule or a set of closely related isomers as a mathematical construct of certain specific and repeating structural groups. A complex mixture such as petroleum can then be represented as thousands of distinct molecular components, each having a mathematical identity. This enables the automated construction of large, complex reaction networks with tens of thousands of specific reactions for simulating the chemistry of complex mixtures. Furthermore, the method provides a convenient framework for incorporating molecular physical property correlations, existing group contribution methods, molecular thermodynamic properties, and the structure-activity relationships of chemical kinetics in the development of models [Quann 1998].

Mathematical Modeling Tools

Factorial Design Studies

The most common strategy for studying mixed exposures is to determine the effects of combinations of two particular exposures using a simple factorial exposure matrix. This strategy focuses on whether the combination of two exposures yields greater or lesser than additive effects compared with the single exposures. An appropriate biological system is exposed to exposure A, exposure B, or the combination of A+B, often accompanied by a sham control exposure. This is the most direct approach for testing hypotheses about specific two-factor interactions and mechanisms of interactions. A simple factorial design can be applied in experiments using response models ranging from very simple, short-term assays (for example, cells) to long-term, complicated models (for example, life-span carcinogenicity or noncancer animal bioassays). However, this strategy is seldom used for exploring interactions among more than two or sometimes three exposures. When toxicity studies become too complex, a step-wise approach may be used. For example, if whole response surfaces are to be studied for mixtures

of five or more chemicals, factorial designs become too complex. To manage this situation, simplified statistical designs, for example, fractional factorial designs, can be used as a starting point to study deviations from additivity. Thus, the response surface analyses can be economized [Groten et al. 1997].

Isobole Analysis

An isobole is a counter line that represents equivalently effective quantities of two agents or their mixture and was used as early as 1870. A hypothetical straight line can be developed for additivity and concave lines (upward for synergism and downward for antagonism) for interactions. The isobole approach is widely used to evaluate the effects of binary mixtures. This method is tedious and tends to produce large standard deviations. The approach also requires large data sets, the precise doses of each of the components in the mixture, and the existence of extensive studies with the single compounds to yield reliable results. In addition, the analysis can be done only when clear effects levels are observed [Cassee et al. 1998].

A similar approach constructs theoretical dose-response curves for dose-additive and independent combinations of components and compares them to the observed response. Although this method requires less data and is more straightforward than the isobole approach, it still requires complete dose-response curves with fixed concentrations and statistical interpretations [Cassee et al. 1998]. A related approach is response surface analysis, which sometimes allows more rapid analysis of the toxicological effects of mixtures with many fewer animals.

Zero-interaction response surfaces describe dose-response relationships for which no interactions between multiple exposure concentrations exist. They define zero interaction according to a particular criterion throughout the complete dose range. This means that they can replace the tedious experimental determination of dose-addition isobolograms (isoboles are specific cross sections of response surfaces). They predict expected combination effects from single-agent dose-response relations but not combination effects that are not zero-interactive. Response surface methods have been incorporated into a number of commercially available computer programs, such as CombiTool [Dressler et al. 1999].

In addition to their application in the design of experiments (see above), response surface techniques can be used as a visualization tool to elucidate the kind and extent of component interactions. Experimental data can be directly compared with zero-interaction response surfaces to assess the likelihood and direction of possible interactions, depending on whether they lie above or below the surface. In addition, response surfaces can be constructed to take into account an interaction mechanism (hypothesis) and can thus be used for exploring the validity of hypotheses for mixtures interactions.

A major limitation of response surfaces is that they readily represent the combined effect of only two compounds or classes of compounds, although the possibility exists of using similar methods to visualize interactions of a particular chemical with the rest of the (complex) mixture as a whole.

Other Mathematical and Statistical Tools

Other approaches are also being developed to identify the structural classes of chemicals and combinations of chemicals within complex mixtures that contribute most strongly to biological effects. Several are adaptations and variations of multivariate analyses [Eide

2001]. For example, regression modeling of mutagenicity data from highly complex, petroleum-derived mixtures of polycyclic aromatic hydrocarbons (PAHs) using partial least squares projection to latent structures was found useful for associating mutagenicity with a limited number of chemicals and predicting responses to other mixtures [Eide et al. 2001].

Rapid development is occurring in the modeling of highly complex biological data, driven in part by the tremendous volume and complexity of data produced by contemporary genomic/proteomic technologies. The bioinformatics field is developing rapidly, and many of the resulting data displays and analytical strategies aimed at identifying response associations (that is, cluster analyses) will be useful in research on mixed exposures. Many of these techniques use visualization of graphical response surfaces.

New Knowledge Needs

- Achieve better understanding of the toxicology (biological mechanisms) of mixed exposures at doses relevant to current exposure concentrations.
- Develop new methods to understand and integrate experimental data from the molecular to the whole organism level.
- Develop the ability to use data from proteomics or genomics studies and extrapolate these to whole-body systems.
- Develop and validate mechanism-based models and predictive tools for use in improving current risk assessment processes for mixtures.
- Develop improved cell models for large-scale studies of the nature of chemical interactions that lump responses by chemical classes.
- Improve statistical tools to identify mixed effects from available epidemiological data.
- Develop the concept of the *virtual human* via PB/PK simulation.

Potential for Intervention

- Understanding and predicting precursors of adverse effects will lead to earlier and more effective intervention strategies.
- Improved forecasting of interaction effects from mixed exposures using less costly, cellular-based screening tools and computer modeling.
- Improved risk assessment and mitigation or intervention methods.

Exposure Analyses

Risk is estimated by integrating a health assessment and an exposure assessment, thus making high-quality exposure assessments essential. Achieving this requires improvements and the integration of methods, measurements, and models for exposure. Research in these areas has been recommended or is under way for individual stressors [NIOSH, 2002]; and in some cases, the findings can be applied directly to mixed exposures. However, in many cases, mixed exposures present a unique challenge. Exposure analyses need to be incorporated into epidemiological studies to obtain robust associations or to eventually achieve knowledge of cause-effect relationships. Knowledge of exposure needs to be applied to the design of more realistic animal toxicological studies. Better exposure analysis

is needed to estimate the number of people exposed to mixtures, the agents to which they are exposed, and the duration and time-course of the exposure. From these estimates, dose-response models for complex mixtures may predict adverse effects. Exposure analyses also identify the sources and pathways most likely to contribute to risk, facilitating efficient and effective interventions.

Methods

Methods enable measurements, making methods of fundamental importance. When contemplating what methods are needed, the goal of the exposure analysis must be kept in mind because this drives the approach. For example, is the goal associated with routine monitoring to identify releases or low-level frequent events? Is it part of an epidemiology study and if so, how quantitative does it need to be? Is it part of a survey, how quantitative does it need to be, and does the source need to be identified? Does the exposure analysis need to be highly quantitative or are there surrogate indicators or questionnaire items that are adequate?

Complex Chemical Mixtures

Measuring single chemicals and complex mixtures of chemicals have similar elements. However, complex mixtures present additional challenges, especially in collecting samples, preparing and extracting samples for measurement, and measuring the compounds present. The first step, collecting a representative sample, is quite difficult primarily because the inherent variability and heterogeneity of mixtures (chemically, physically, spatially, and temporally) create challenges. For example, a sampling method optimal for an aerosol mixture rich in nonreactive hygroscopic materials is different from one rich in nonreactive volatile organic compounds. This problem calls for the development of new sampling technologies capable of sampling mixtures with improved precision and accuracy that are as representative of the original environment as possible. Obtaining a representative assessment of a worker's exposure is complicated further when the potential exists for absorption through the skin. Ultimately, a form of biological monitoring; for example, of blood, urine, or exhaled breath, may be required to estimate a worker's total exposure by all routes.

The next stage is preparing the collected sample for measurement. Analytic procedures for pure chemicals in a pure water or simple organic matrix are highly developed. However, environmental and occupational matrices are complex. For example, a procedure that works well with a breath sample, will likely not work well for an aerosol sample. Consider the case of extracting a mixed sample with chemicals varying widely in solubility and other physicochemistries.

The final step is to take the measurement. Newer technologies, whether they are biologically (as in biosensors or biomarkers) or chemically based (as in more advanced mass spectometry technologies growing from traditional analytic chemistry approaches) can also be quite difficult for mixtures. In spite of major advances, some classes of substances are still difficult to identify quantitatively. For example, only a very small fraction of the organic components of ambient aerosols have been chemically identified. Thus, it may be possible to measure only a portion of the mixture. If this portion causes the health effects, this is acceptable. However, false negatives might result, weakening reliance on such procedures.

The analysis of complex mixtures of substances is still a daunting task. High-resolution

chromatography and mass spectrometry are examples of current techniques commonly applied to the analysis of complex mixtures. Combining such technologies to form multidimensional techniques provides even more powerful analytical tools. For example, gas chromatography-mass spectrometry (GC-MS) is well established for characterizing and quantifying the various volatile chemicals that constitute mixtures such as petroleum distillates. Techniques for characterizing and quantifying the nonvolatile, polar, and thermally labile components of complex mixtures are less well developed and need to be elaborated.

The broad application of sophisticated analytical technologies to the analysis of complex mixtures is hindered by the expense of the equipment and the need for highly skilled operators. Also, the time required for chromatographic analysis, which is based on differences in partitioning of the various sample components between a mobile and stationary phase, increases with increasing mixture complexity. The development of new technologies, such as microsensor arrays, holds the promise of providing rapid, specific responses to a variety of significant endpoints, such as those based on electrochemistry and immunochemistry.

Measurements/Monitoring

Various approaches can be used to measure exposures. First, emphasis needs to be on deciding the exposure metrics. This includes identifying the full spectrum of stressors being measured (for example, inhaled chemicals, noise), the time frame (for example, exposures to chemicals having acute effects or peak-exposure effects should be measured over short averaging times), organization of work issues (such as extended or novel work schedules), and identifying data quality objectives (for example, precision and accuracy needed, sample size needed). However, given the state of the science, these elements are often difficult to determine. Without more knowledge of exposure variability, it can be difficult to decide on an optimal measurement strategy. For example, is it better to measure a large number of workers once or a small number of workers frequently? Another key question is whether a stationary monitor can adequately represent worker exposures or are personal exposure measurements required. Most likely, the answer depends on the exposure scenario. This must be determined in advance so that the optimal approach can be chosen.

Exposure Modeling

All exposure scenarios cannot be measured for reasons such as limited finances and limited availability of measurement methods. Hence, exposure modeling is necessary. Optimal models are built using a combination of measurement data and theoretical information and are evaluated with measurement data. Modeling becomes even more important with mixtures because of the difficulty (and in some cases the impossibility) of measuring complex mixtures. For example, exposures could be better predicted if a complex exposure model were available based on the chemicals in the environment of interest; the physiochemical properties of the chemicals; the relevant fate, transformation, and distribution characteristics under realistic conditions; and activity patterns of the potentially exposed people. With a scientific basis to estimate the number of people likely to have exposure to other stressors (for example, noise, certain pharmaceuticals), the total exposure would be better understood as input into health models for eventual risk assessment.

A need exists for research on mixed exposures that (by virtue of their chemical or

physical properties) react in the work environment (before entering the body), producing a more hazardous chemical or resulting in greater ease by which the agent enters the body. For example, a mixed exposure involving ultraviolet light and certain chlorinated hydrocarbons can produce the toxic agent phosgene [Ng et al. 1985; Wang et al. 2002]. Another example is the mixed exposure involving fine particles and radon gas that can result in increased lung burden of alpha and beta radiation emitters.

New Knowledge Needs

- Develop and validate new sampling technologies with defined accuracy and precision, especially with respect to representativeness to the environment being sampled.
- Develop biosensors (microsensor arrays) or measurement technologies that provide direct indication of toxicity (biological relevance) of mixed exposures.
- Develop improved separation and sample preparation procedures.
- Develop a protocol for determining the adequacy of a questionnaire approach or a surrogate indicator approach to exposure analyses of mixtures. Provide criteria to characterize the adequacy of such an approach.
- Develop more portable, rapid, automated, affordable, and sensitive methods for measuring chemical mixtures, including screening methods for classes of chemicals.
- Understand major exposure variability factors that are critical components of measurement strategies.
- Develop models of exposure to complex mixtures that include environmental transformations that alter the toxicity or change the uptake of the agent by the body.

Potential for Intervention

- Better exposure assessment methods will improve recognition of problems.
- Understanding environmental interactions can assist in devising practical solutions.
- Monitoring exposures will determine whether the solutions were effective.

Biomarkers

The term *biomarker* is used quite broadly to refer to indicators of exposure, effect, or sensitivity that are measured in biological samples or systems. Biomarker measurements have a high potential value because they are made with human samples; whether that value is realized is a function of how well the biomarker is understood. For example, some people interpret the presence of a chemical in blood as an indication of an adverse effect. For some well-studied chemicals, such as lead or carbon monoxide, blood levels can be equated to different degrees of health risk. However, for most chemicals of interest, the methods to accurately measure the biomarker and the relationships of a biomarker to effects and to sources of the chemical are unknown. Some measurable biomarkers may be indicators of a health effect, while others are simply indication of past or present exposure. Similar examples could be given for biomonitors of health, whether they are relatively simple (for example, a symptom questionnaire) or com-

plex (for example, full medical exam). Deoxyribo nucleic acid (DNA) adducts have been studied for years, but what does the presence of a certain level of DNA adducts mean?

Genomics is rapidly enabling the development of more information about a person's genetics, but it will still be necessary to determine, for example, whether a particular genetic array of metabolizing enzymes places a worker at higher risk. Genomics, proteomics, and other related technologies could also be useful to screen for changes in gene expression in persons working in one environment versus another. Although the literature on promising biomarkers is growing, the ability to interpret them in terms of health risk and prevention is not nearly commensurate; and there are significant ethical issues related to obtaining and applying genetic data.

Applying the concept of biomarkers to studies of exposures to complex mixtures can greatly aid in understanding the consequences of such exposures and may help identify the active components. These issues can be difficult when studying specific agents but become even more complex as the exposure complexity increases. However, the advantage of applying biomarker data is that a finite number of responses or health outcomes may be categorized. By carefully working backward from the response to the exposure, it may be possible to identify events, markers, or changes that can be monitored or used to predict outcome, or used to design prevention plans. Exposures for which identical metabolic intermediates are produced could provide useful mechanistic information.

Most risk characterization approaches for mixtures rely on estimations of risk of a few components of the mixture. This creates significant uncertainty and variability. By directly measuring adequately characterized biomarkers, it is possible to more effectively predict, measure, and intervene in adverse health events.

New Knowledge Needs

- Identify, develop, validate, and characterize the health outcome for biomarkers of exposure and response for workers exposed to mixtures.
- Identify chemicals that produce identical metabolic intermediates in the human body for priority study.
- Develop methods to measure in biological media a larger number of chemicals and other stressors likely to be related to mixed exposures.
- Develop data from a national human exposure survey that focuses on exposure and its relationship to effects and the sources of exposure that may lead to risk. Because a suite of chemicals would be studied, many real-world mixtures would be identified.

Potential for Intervention

- Biomarkers are especially important for intervention strategies for mixtures since they are rarely fully measured and are rarely fully understood in terms of risk. By being the biological *integrator* of mixtures exposure and effects, such markers could be useful if validated to show they are integrating accurately across mixtures.
- Biomarkers that are interpretable can be applied to identify (1) the need for intervention (for example, a risk has been identified), (2) the persons in the group who may be more affected

or more susceptible and therefore need to be followed closely, and (3) the effectiveness of the intervention (for example, Did reducing source emissions or using protective gear reduce effects?).

Risk Assessment Methods

A pre-eminent need in the field of mixed-exposure research is to develop scientifically valid risk assessment strategies that can facilitate establishing protective regulatory standards and risk management procedures. Ideally, to determine the toxicity of any chemical mixture, one would determine the range of possible exposures and test the complete mixture for these exposures. Traditional risk assessment of individual agents or stressors has relied on toxicological tests such as the 2-year rodent bioassay, a laborious and expensive procedure. With the infinitely large number of chemical mixtures in the environment, risk assessment methods that rely on the conventional methodologies and approaches are not feasible because of the immense resources required. The challenge for mixed-exposures risk assessment is to develop alternative methods that can take the data available for chemicals or mixtures and make scientifically valid predictions for priority mixtures of relevance to occupational and environmental exposures.

Risk assessment for mixed exposures is limited by the availability of data. To meet the mandate and limitations imposed by various laws, individual chemicals, stressors, or biological agents have been tested, and one rarely finds studies that have data on evaluation of multiple health effects in the same organism. Thus, studies are needed in which multiple health effects have been assessed in the same animal or human populations, representative of real life epidemiological studies.

One approach that could be applied to complex mixtures of varying composition is to identify all the chemicals in a mixture, determine the toxicity of each, and have available complete information regarding the possible interactions of all components of the mixture over the expected exposure ranges. For complex mixtures, data are typically inadequate to make predictions with certainty: not all components of the mixture may be identified, the proportions of components may not be known, information about possible interactions between components will be rare, and epidemiologic data on human health effects are often missing. Risk assessment in the area of chemical mixtures, therefore, is characterized by making judgments in the face of multiple unknown factors.

Whole-Mixture Approach (Mixture Treated as a Single Toxic Agent)

Whole-mixture testing considers the mixture as a single entity and conducts a standard health risk assessment for the chemical mixture in the same way that one is conducted for a single chemical. It is the simplest way to study the effects of a mixture, because the sole information needed to apply this method is the dose-response curve of the whole mixture in the organism desired.

Dose-response data on the whole mixture as a single entity are ideal for risk assessment because the extrapolations are minimal. This method has been used for the risk assessment of cigarette smoke, diesel exhaust, and mixtures of groundwater contaminants. Influences of possible interactions among the components of the mixture are included because the whole mixture has been tested. However, this approach cannot identify any toxicologic

interactions or the causal mechanisms for the observed toxicity. Whole-mixture procedures are best for mixtures that maintain fairly constant composition and exposure concentration throughout the expected timeframe of the exposure. Dose-response data for whole mixtures, however, are rarely available, in part because most legislative promulgations have been oriented toward single-chemical exposures. Even if such data are available, extrapolation across routes or from high to low dose may be required, introducing uncertainties. No information about the identity of the mixture components is obtained, and testing of all relevant potential mixtures (for variations in dose or proportions) is impossible.

Similar-Mixture Approach

The similar-mixture approach uses data on a well studied, but toxicologically similar mixture to estimate the risk from the mixture. Mixtures are usually judged to be toxicologically similar based on composition or observed toxicological properties. Mixtures may have the same components at different ratios, some common components but some unique ones, or one or more additional components when compared with the original [51 Fed. Reg.† 34014(1986); EPA 2000]. The main toxic effects should be the same for the surrogate and mixture.

Group of Similar-Mixtures Approach

A different kind of similar-mixture approach is called the comparative-potency method [Lewtas 1985]. In this approach, the human toxicity of the mixture is estimated from that mixture's toxicity in a nonhuman study by multiplying by a proportionality constant that is estimated from data on the other mixtures in the similarity set. This approach is empirical, requiring human dose-response information for the main adverse health effect for a mixture that is toxicologically similar to the one in question.

Although this approach includes interaction effects, it focuses on only one health effect, so it may not provide an adequate evaluation of the overall health risk. In addition, full information about similar mixtures is rare, so this approach may not be readily useful in most risk assessment situations. This method has not been used extensively, so its general validity and applicability are still undetermined. It has primarily been used with carcinogens.

Component-Based Mixture Approaches

A single component of a chemical mixture may be a relevant index of toxicity when that component is suspected to account, qualitatively and quantitatively, for most of the toxicity. For example, photochemical pollution is typically indexed by the level of ozone, the principal oxidant. The concentration of ozone is used for both health and regulatory purposes with regards to the mixture, and ozone levels are widely used in toxicologic investigations as a surrogate for the mixture sample. Another example is the use of benzo-pyrene as an indicator for the carcinogenic potential of mixtures of PAHs. This approach is useful, under the appropriate conditions, because only the dose-response information for the indicator is required. Ideally, a marker of exposure to a complex mixture should be (1) unique to the mixture in context, (2) present at a consistent ratio to other components, (3) readily detectable at lower concentrations, and (4) measurable with good accuracy at a reasonable cost. Obviously, the disadvantage of this approach is that the potential toxicity of other mixture components is ignored.

†*Federal Register.* See Fed. Reg. in references.

Mathematical Models of Joint Toxicity

Component-based risk assessment of simple, identified mixtures can be improved by using physiologically based models reflecting PK/PD of the component chemicals. Risk estimates can be tailored to exposure situations and worker characteristics by incorporating time-dependent exposure patterns and physiological factors appropriate to the situation. For such modeling methods to be implemented as standard practice, a set of central and health protective default parameters must be used in the absence of chemical-specific data.

Risk characterization from PK/PD models should include a description of the uncertainties. Parameters in these models are rarely measured independently, so that biologically based models usually include default or estimated parameters. Uncertainties in the risk application should then address model fit, judgments of relevance of supporting data (if extrapolation is used), and biases (for example, use of protective assumptions or confidence limits).

Toxic Equivalency Factors

This approach is appropriate when the components of a mixture are all congeners or isomers of the same chemical and have been used extensively for risk assessment with PAHs, halogenated aromatic hydrocarbons, and endocrine disruptors. It is used to provide an estimate of the potency of less well studied components in a mixture relative to the potency of a component that has undergone more extensive testing, termed the index chemical. Each component's exposure is converted into the toxicologically equivalent exposure of the index chemical by scaling by the relative potency. The scaling factor is called the toxicity equivalence factor (TEF). The mixture exposure is then calculated by summing these equivalent exposures to obtain the mixture exposure in terms of the equivalent index chemical exposure [EPA 2000].

This approach requires complete knowledge of the mixture composition and assumes that all components act through the same biologic pathway, the exposure concentrations of the individual chemicals are additive, the dose-response curves for different congeners are parallel, and the chemicals act on the same organs over the doses studied. Using EPA's definitions of interaction, this approach then assumes no interactions between isomers and that a single TEF is valid for all types of toxic effect. The principal use of this approach in occupational exposures has been in investigations of mixtures of volatile organic compounds, often a suspected culprit in incidents of sick-building syndrome, and for mixtures of dioxins.

Hazard Index (HI)

Risk assessment of mixed exposures often combines pieces of information that differ widely from each other. Exposure data for some stressors may be only as time-weighted averages, while others reflect daily activity patterns. Toxicity data for some chemicals may allow estimation of probabilistic risk for one endpoint, while only providing qualitative descriptions of other endpoints. It is possible to develop the risk characterization using the original information in a high-dimensional matrix, but such a summary will be difficult to evaluate and communicate. One approach to diverse multivariate facts is the decision index, used as an action level for regulatory action or occupational health intervention. The advantage of a decision index is the simplicity in converting highly multivariate technical information into a single number. The most common example used for health risk is the HI for mixture risk.

Although specific for a single affected target organ, each HI is based on multiple studies of multiple chemicals, often involving multiple-test animal species and test exposure concentrations and highly varied measures of toxicity. The HI is a rough implementation of dose addition, in which all component chemicals are assumed to be toxicologically similar. The HI is the sum of single chemical exposure concentrations, with each scaled by its relative toxic potency, most often implemented by using the ratio of the exposure concentration to the corresponding acceptable concentration (for example, TLVs for long-term exposures or short-term exposure limits [STEL] for short-term exposures), commonly called the hazard quotient.

This summation of scaled component concentrations has its regulatory origin in the ACGIH formula that was adopted by the ACGIH in 1963. This was incorporated into OSHA regulations [29 CFR 1910.1000 (d)(2)] shortly after passage of the Occupational Safety and Health Act of 1970. By the ACGIH formulation, the additive hazard index approach is only used when substances act on the same organ system [ACGIH 2003]. Note again, that the ACGIH criterion of *same organ system* is somewhat different from the criteria described by the Food Quality Protection Act, that the components have the same *mechanism of toxicity.*

The version of the HI developed by the EPA Superfund Program Office is by far the most common approach used in conducting mixture risk assessments in the field, aided in part by the ready availability of reference doses for oral exposures and reference concentrations for inhalation exposures, which are used for the scaling factors. When the calculated HI value for a mixture exceeds 1, it reflects a health risk similar to that involved if an individual chemical exceeded its concentration limit by the same extent. The EPA recommends that a separate HI be calculated for each toxic effect concerned [EPA 2000]. In addition, Feron et al. [1995a] proposed combining a hazard hierarchy scheme with the HI approach by selecting the top 10 chemicals in a complex mixture with regard to toxicity, and then combining the relative toxicities of each into a single measure of relative risk for the mixture.

The main disadvantage of a simple index is that the uncertainties in its calculation are largely hidden. Another key disadvantage is in quantifying what are often scientific judgments. For example, the HI implemented under Superfund is a number whose decision threshold is usually given as 1.0, so that when HI>1, additional action is indicated. A numerical estimate of the uncertainty in the HI value would help interpret the need for additional action.

Target Organ Toxicity Doses

The use of an acceptable level in the relative toxicity scaling factor (for example, 1/TLV or 1/reference dose [RfD]) may be overly protective in that the RfD (or reference concentration [RfC]) is based on the critical effect, defined as the toxic effect occurring at the lowest dose. When the HI is calculated for a different, less sensitive effect, the RfD will be too low, so the factor (1/RfD) will overestimate the relative toxicity, and the HI will be too large. One alternative that avoids this critical effect conservatism is to use a toxicity-based exposure concentration that is specific to the target organ and is derived similarly to an RfD (or RfC). For oral exposures, this value is called the target organ toxicity dose (TTD) [Mumtaz et al. 1994]. The formula for the HI would be identical, with the TTD replacing the RfD. For inhalation exposures, a similarly defined target organ toxicity concentration (TTC) could be used. This same

approach can be applied to HIs for shorter exposures by using the effect-specific data appropriate to the shorter exposure period of concern.

The TTD is not a commonly evaluated measure, and no official EPA activity is deriving these values as for the RfD and RfC. This alternative should be considered when there is sufficient reason to believe that the overestimate of the HI caused by use of RfDs is significant to the interpretation of the mixture assessment. In that case, TTDs can be derived for the mixture components by following the scientific steps used in deriving an RfD. The evaluation of quality of the candidate toxicity studies and the choice of uncertainty factors should parallel those steps in the RfD process. One difference in the uncertainty factors concerns the factor for completeness of the database used for RfD development. For example, if no two-generation study existed for a chemical, there could be an additional uncertainty factor used to obtain the RfD, because the RfD must protect against all toxic effects.

However, when a renal TTD is developed, no additional factor would be used because the data would only include renal effects.

Any TTDs derived for a mixture assessment must be clearly documented, including the array of studies considered, the study and dose selected for calculation, and the uncertainty factors chosen. When the critical effect of a chemical is the effect being described by the HI, the RfD and TTD will apply to the same target organ and so should be the same unless the TTD is based on newer information. When data for one or more components are not sufficient for deriving their organ-specific TTDs, their RfDs should be used and noted as a source of possible overestimation of the HI. These recommendations and discussions also apply to HIs for shorter exposures and to TTCs as replacements for RfCs in an HI for inhalation exposures.

Estimation of Interactions

This approach attempts to characterize synergism or antagonism in a mixed exposure based on putative interactions between the components. Effect modification is considered to have occurred when the combined effect of two or more exposures is larger or smaller than the anticipated effect predicted by the exposures individually. Strict criteria for using and evaluating this approach have yet to be developed; statistically significant data will require very large numbers. It has, however, been effectively used to study the combined effects of agents known to be independent risk factors for disease, for example, cigarette smoking and asbestos.

Better laboratory and analysis tools for identifying synergism and antagonism are needed. Testing of more contemporary chemicals is also needed. The combined synergistic effect of environmental chemicals with regards to endocrine disruption has not been adequately studied. There is also a need to better define the concepts of synergism and potentiation and to raise awareness of varying mathematical concepts of additivity [Simmons 1995].

Interaction-Based HI and the Weight of Evidence (WOE)

The HI approach does not account for interactions that may occur within the mixture. Toxicologic interactions have been mostly studied with binary mixtures. One way to include interactions in a mixture assessment is to modify the noninteractive assessment by knowledge of these binary interactions; a tacit assumption is then that higher order interactions are relatively minor compared with binary interactions. Although some mix-

ture data exist [Lof and Johnson 1998], few studies quantify interaction, and even fewer quantitatively describe the dose-dependence of the interaction. Consequently, for an approach to be able to use available data, some qualitative procedure is needed for judging the impact of the potential toxicologic interactions. The WOE approaches used by the EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) are an attempt to qualitatively combine empirical observations and mechanistic considerations [Mumtaz and Durkin 1992].

The WOE approach determines the most plausible influence of one chemical component on the toxicity of another chemical in the mixture for a given exposure scenario. Factors in the determination include the direction of the interaction (adverse effect, greater than, less than, or equal to additive effects), mechanistic support (how an interaction might have occurred), toxicological observations (directly demonstrated, inferred from a related compound, or unclear), modifying factors (exposure sequence and/or duration), limitations, uncertainties and references [Hansen et al. 1998]. Confidence in the prediction is greater when the mechanism by which the interaction may occur is well characterized. A rating matrix can also be developed when the other determinations are made. Binary mixtures will have comparatively simpler ratings than a mixture with more components and a complex rating matrix. The WOE is potentially useful for a variety of chemical mixtures, but it is an approach that has not been adequately validated by experimental or epidemiological data.

The interaction-based HI uses the WOE approach to modify the HI calculation. The EPA procedure modifies an earlier method [Mumtaz and Durkin 1992]. The WOE determinations are converted into numerical scores, and then combined with functions of the component exposure concentrations and the component hazard quotients to give an HI that incorporates the pair-wise interactions. The formula is modular so that improved dose-interaction relationships can be easily incorporated. Published interaction information, however, does not usually include interaction magnitudes, only the direction of interaction (for example, synergism) so that evaluating the accuracy is difficult. The HI-WOE method works only when the HI approach works. The HI approach is based on the principle of additivity. The principle of additivity is recommended for those toxicants that have similar mechanisms of action. Thus, the HI-WOE approach works for toxicants with similar mechanisms of action and not for dissimilar mechanisms of action [Mumtaz et al. 1998].

Occupational Exposure Limits

Few mixed-exposure regulatory standards have been established because assessment methods for mixed exposures have been based on extrapolation rather than direct toxicological data [Mumtaz et al. 1995]. The current challenge for environmental and occupational scientists is to provide a sound, scientific basis that enables policymakers to substitute current, simplistic, single chemical standard setting with real-life, mixture-oriented standard setting [Feron et al. 1995a]. The European Maximum Workplace Concentration (known as *Maximale Arbeitsplatz Konzentration* or MAK) Commission refrains from setting scientifically based occupational exposure concentrations for complex mixtures because they feel that meaningful standards are not possible given the inadequacy of the data [Bartch et al. 1998].

Another approach for regulating exposure to mixtures is contained in the OSHA and MSHA Hazard Communication Standards [29 CFR 1910.1200] and [30 CFR 47], respec-

tively. These standards prescribe labeling and worker training for substances with recognized potential for producing health effects in workers. The Hazard Communications rules provide the following logic for dealing with mixtures:

- If the mixture is tested as a whole, use the results of testing.
- If it is not tested as a whole, the mixture

—has the same hazard as any of its components that are present at a concentration greater than 1%, and

—is carcinogenic if it has any carcinogenic components present at a concentration greater than 0.1%.

- If evidence shows that an individual occupational exposure limit has been exceeded, use those data.
- If there are scientifically valid data for physical hazard elimination, use those data.

New Knowledge Needs

- Develop procedures for evaluating the utility and uncertainties of whole-mixture data in risk estimation.
- Develop methods that can use data from specific chemicals or mixtures to enable scientifically valid predictions about unknown mixtures.
- Develop methods to test the validity of risk assessment based on comparative potency analysis.
- Develop methods to test the validity and applicability of the hazard index approach for dealing with occupational exposure settings.
- Develop methods that can estimate the impact of components of whole mixtures in terms of synergism, potentiation, or antagonism, including estimates of interaction thresholds.
- Develop methods to (1) extrapolate interaction results to mixtures with different component ratios, or different total dose and (2) extrapolate from *in vitro* to *in vivo* as well as across routes to estimate risks and protective exposure limits.
- Develop mechanistically based default parameters for risk estimation, including default estimates of pair-wise interaction magnitude as well as PK/PD model parameter distributions for worker populations.
- Compare and evaluate alternative risk assessment methods by identifying and applying standard data sets representative of worker exposures.

Potential for Intervention

- Improved risk assessment data will enable improved risk management decisions and actions.
- Improved methods for risk assessment can be used to assure that correct public health decisions are made when assessing exposure settings.
- Summary index methods may provide a useful approach for controlling exposures in workplaces.

Controls

The preferred methods for protecting workers from hazards are engineering controls (such as ventilation, isolation, and substitu-

tion); administrative controls (such as rotating workers' tasks) are also recommended. Generally, an engineering control will be equally as effective for all components of mixed exposures. However, in instances such as substitution, the choice of the substitute material could create a new hazard by creating a new exposure situation and creating a multiple, nonsimultaneous, mixed exposure with the original agent. Use of administrative controls, such as rotating workers to different jobs or tasks, can create this same scenario of multiple nonsimultaneous exposures. Mixed exposures could defeat filtration controls, for example if an electrostatic filter is simultaneously exposed to humidity, gases, and vapors that affect the filters' electrostatic charge, it may be degraded to reduce its efficiency to filter particles. Mixed exposures often occur in agriculture, construction, and other workplaces where engineering controls are not feasible. In these workplaces, personal protective equipment (PPE) is often used.

The use of respirators and biological and chemical protective clothing for protection against mixed exposures presents a unique challenge. First, mixtures can have an effect on the efficiency of air-purifying respirators. Gases and vapors can degrade electrostatic filter media, decreasing filter efficiency. Gases and vapors trapped on the surface of a particle can off-gas from the filter media where they are captured. Combinations of gases and vapors can decrease service life on a cartridge or—even in cases for which adsorption of one gas/vapor is preferential—render the cartridge useless against other gases/vapors in the mixture. Studies on chemical protective clothing have indicated that breakthrough times at one contaminant concentration of a mixture is not predictive of the service lives of the clothing when different concentrations of the component are present. Also, studies of chemical mixtures on biological and chemical protective clothing can show a synergistic effect on breakthrough times [Mickelsen and Hall 1987; Mickelson et al. 1986].

Additionally, protective equipment can create a mixed exposure. Respirators and biological and chemical protective clothing both can cause physiological and psychological stress for the wearer. In fact, the most protective gear may present the greatest set of stressors. Self-contained breathing apparatus can weigh up to 35 pounds and increase workloads up to 20 percent. Heavy protective clothing, such as firefighter ensembles, can increase heat stress and the worker's cardiac demand. Psychological responses to respirators and full body ensemble responses include phobias such as claustrophobia.

In 1998 in Chicago, the Control Technology and Personal Protective Equipment NORA Team held a workshop—Control of Workplace Hazards for the 21st Century. Several of the knowledge needs identified apply to mixed exposures. The workshop recommended the following:

1. Perform research to determine change-out schedules for cartridges and filters. This is particularly important for mixtures in which the presence of one contaminant will affect the ability of respirators to protect against other contaminants in the air. Four methods should be explored: laboratory testing, work place testing, sensors (for example, end-of-service-life indicators), and mathematical models.

2. Evaluate physiological and psychological responses to workplace tasks and the wearing of respirators in order to minimize stressors. The evaluation should consider interaction with oxygen uptake, dead space carbon dioxide concentrations, comfort, effects on hearing

and communication, thermal stress, and phobias.

3. Develop state-of-the-art monitoring equipment and technologies that can be used in conjunction with PPE for mixtures. For example, improve laboratory and field testing methodologies including establishing end-of-service-life indicators and advanced real-time biological and chemical monitoring technologies such as micro sensors, colorimetric techniques, analytical techniques, and field detectors that are effective in mixed exposures.
4. Investigate physiological and psychological factors associated with the PPE and the environment to reduce the effect of mixed stressors. For example, investigate practices for reducing the impact of heat stress or extreme cold.
5. Explore decontamination procedures that are effective against mixtures.

New Knowledge Needs

- Determine the effect of mixture components (including nontoxic engineering controls and PPE, that is, the potential effect on the efficiency and durability of the factors such as humidity) on equipment against other toxic components in the mixture.
- Determine the physiological and psychological stresses generated by PPE and potential interaction with exposures that result in spite of or because of using the PPE.
- Develop and evaluate decontamination methods for engineering controls or PPE used as protection from mixed exposures.

Potential for Intervention

- Appropriate engineering controls or PPE will be selected and used against mixed exposures.
- Stressors from the use of PPE will be minimized.
- Manufacturers will have an incentive to produce improved engineering controls and PPE that are effective for reducing or eliminating exposure to mixtures.

3 Priorities for a Research Agenda Ê

The preceding sections have outlined research needs for hazard identification, effects studies, exposure assessment, biomarkers, risk assessment, and controls. Because research funds are limited, and many worthy needs have been identified, the NORA Mixed Exposure Team recommends the following research needs as among the highest priorities. These priorities were developed based on the following criteria (evaluated using the background in the preceding sections):

1. The lack of available data, with research not elsewhere supported.
2. The possibility that research would make a difference for worker protection.
3. The research should improve our basic understanding of mixed exposures.

On the basis of these criteria, the NORA Mixed Exposures Team recommends the following high priority research topics (NOTE: This list is **not** in a priority order.):

- Develop and implement new surveillance methods to determine the number of workers exposed to specific mixtures, identify the range of exposure concentrations, and identify health effects associated with mixed exposures.
- Develop research strategies that promote collaboration between occupational health professionals and workers in ranking and characterizing mixed exposure within specific occupations and industries. Such assessment will also facilitate dissemination of research findings.
- Conduct research to better understand the toxicology (biological mechanisms) for mixed exposures.
- Develop methods to understand and integrate experimental data from the molecular to the whole organism level. For example, developing the ability to use data from proteomics and/or genomics studies and extrapolate these to whole body systems.
- Develop methods that can be used to measure and predict deviations from additivity.
- Develop and validate mechanism-based exposure response models.
- Develop the concept of the *virtual human* via PB/PK simulation.
- Develop *default parameters* for mechanistically based risk estimation and extrapolation models.
- Develop biosensors or measurement technologies (such as micro-arrays with advanced signal processing) that indicate whole mixture toxicity.
- Identify, develop, validate, and characterize the health outcome for biomarkers of exposure and response, for workers exposed to mixtures.

- Determine the effects of mixtures on engineering controls and PPE; evaluating the mixtures' potential to adversely affect the protection provided by the controls.

Clearly, awareness is growing both in the United States and worldwide of the need for meaningful research on the toxicology and health risk assessment of mixed exposures, not only in the occupational context but for environmental, pharmaceutical, and clinical exposures as well. A strong commitment of resources, as well as deliberate strategic planning, is required to define research agendas and design studies that will effectively provide for risk assessment and reduction of mixed occupational exposure hazards.

When studies are proposed within the priority areas outlined above, or for any of the other research needs described elsewhere in this report, the following factors should be considered in selecting the test mixtures:

- Ability to detect interactions or joint toxicity, that is, beyond that predicted based on individual component toxicity;
- Number of affected workers who will benefit;
- Severity of combined effect under study.

Application of these principles should help avoid the promotion of studies of specific binary mixtures that have limited utility for advancing the science or improving prevention and interventions for many workers but have some particular academic interest.

Conclusions

The topic of mixed exposures is broad. The vast number of permutations, considering studies of binary mixtures alone is astronomical. To promote long-term progress in understanding the basic principles of action for mixed exposures, the team hopes these priorities will lend focus to a very complex area.

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Appendix Glossary of Key Mixed Exposure Terms* Ê

To facilitate communication, it is essential that the key mixed-exposure terms are defined. The need for agreement of definitions takes on added importance when communications among several scientific disciplines, numerous agencies (government, nongovernment, U.S. and international), professional associations, and the public are necessary. Many terms require understanding to shape the mixed-exposures research agenda. This Appendix is provided to reduce potential confusion when discussing mixed exposures and this report.

The list of terms requiring definitions was generated from a review of the literature. Certain definitions were adapted in an attempt to make them more applicable to occupational health.

Additivity	When the effect of the mixed exposure is equal to the sum of the effects of the individual components. The terms *effect* and *sum* must be explicitly defined. Effect may refer to the measured response or the incidence of adversely affected species. The sum may be a weighted sum (see also dose additivity) or a conditional sum (see also response additivity).
Antagonism	When the effect of the mixed exposure is less than that suggested by the component toxic effects. Antagonism must be defined by identifying the type of additivity (dose or response addition) from which the combination effect deviates.
Chemical antagonism	Refers to a reaction between the components that has formed a new chemical. The toxic effect produced is less than that suggested by the components' toxic effects.
Chemical classes	Groups of components that are similar in chemical structure and biologic activity, and that frequently occur together in environmental samples, usually because they are generated by the same industrial process. The composition of these mixtures is often well controlled so that the mixture can be treated as a single chemical. Dibenzo-dioxins are an example.

*Adapted from Hertzberg et al. 1998.

Chemical mixture	Any set of two or more chemical substances. May also be referred to as a whole mixture or as the mixture of concern. (See also complex mixture and simple mixture.)
Chemical synergism	When a reaction between the components has occurred and a new chemical is formed, the toxic effect produced is greater than that suggested by the components' toxic effects and may be different from effects produced by any of the components by themselves.
Complex mixture	A mixture containing so many components that any estimation of its toxicity based on its components' toxicities contains too much uncertainty and error to be useful. The chemical composition may vary over time or with different conditions under which the mixture is produced. Complex mixture components may be generated simultaneously as by-products from a single source or process, intentionally produced as a commercial product, or may coexist because of disposal practices. Gasoline is an example.
Component	Single chemicals or stressors that make up a chemical mixture or mixed exposure. Chloroform is an example of a component in a disinfection by-product mixture.
Dose additivity	When the effect of the combination is equal to the effect expected from the equivalent dose of an index chemical or other stressor (chemical or other stressor as the basis for standardization of toxicity of components in a mixed exposure). The equivalent dose is the sum of component doses scaled by their potency relative to the index chemical or stressor.
Exposure	Contact of a chemical, physical, or biological agent with the outer boundary of an organism. Exposure is quantified as the concentration (or intensity for physical agents) of the agent in the medium in contact integrated over the time duration of that contact.
Inhibition	Refers to the mechanism whereby exposure to one stresor that alone has no effect on a certain biologic activity reduces the adverse effect associated with exposure to another stressor.
Mixed exposures	Exposures to either chemical mixtures, different substances at different times, simultaneous exposure to multiple substances, or simultaneous exposure to a chemical substance and another stressor.

Multiple exposures	Exposure to chemical substances at different times or simultaneous exposure to more than one chemical substance and another stressor. Simultaneous exposure to chemical solvents and noise is both a mixed and multiple exposure. Daily exposure to benzene is a multiple exposure.
Potentiation	Refers to the mechanism whereby exposure to one stressor that alone has no effect on a certain biologic activity increases the adverse effect associated with exposure to another stressor.
Response additivity	When the response (rate, incidence, risk, or probability) of effects from the mixed exposure is equal to the conditional sum of component responses as defined by the formula for the sum of independent event probabilities.
Simple mixture	A mixture containing two or more identifiable components, but few enough components that the mixture's toxicity can be adequately characterized by a combination of the components' toxicities and the components' interactions.
Synergism	When the toxic effect of the mixed exposure is greater than that suggested by the component toxic effects

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