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Washington, DC 20460

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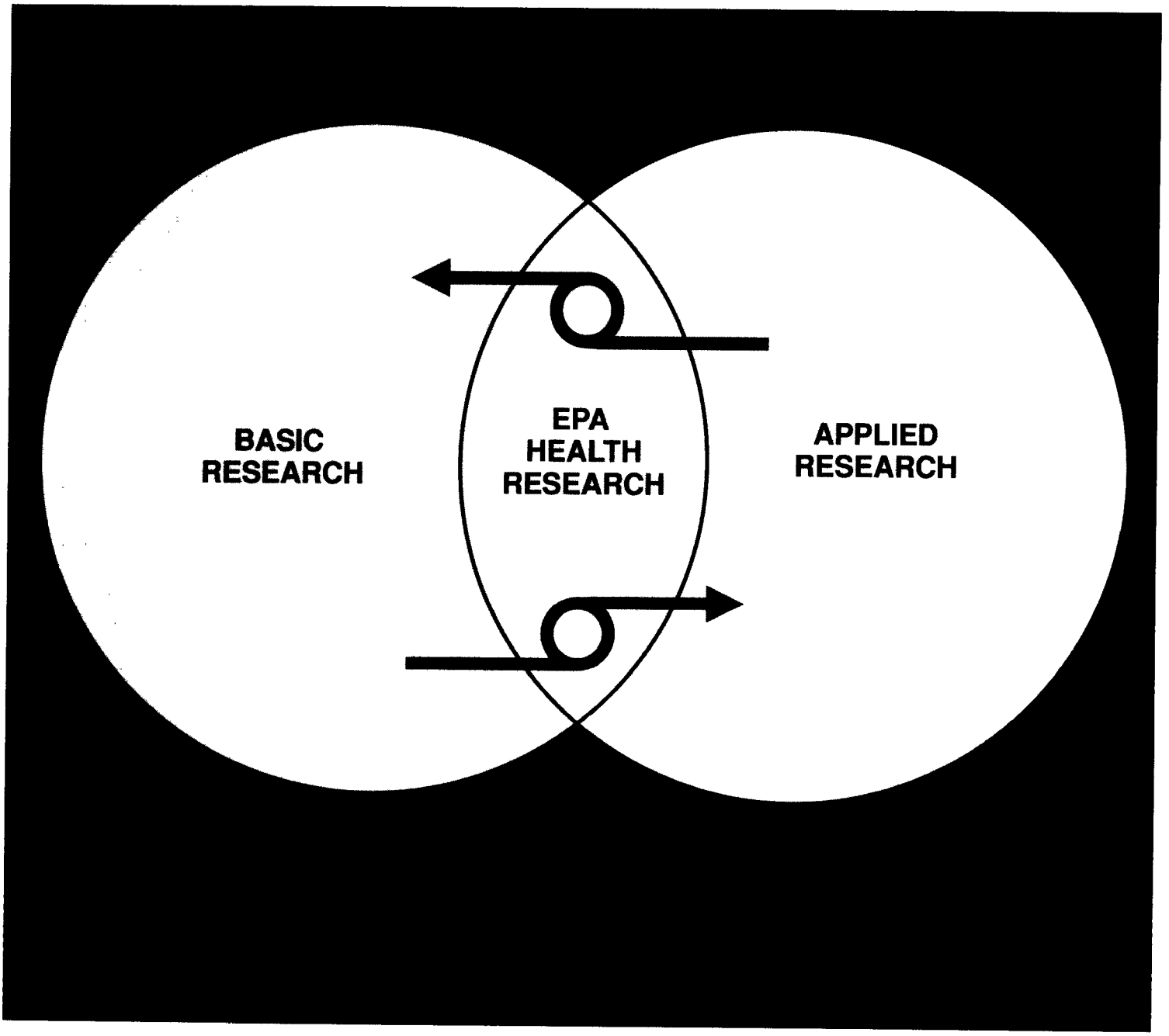
Strategy for Environmental Health Research at EPA



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Strategy for Environmental Health Research at EPA

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FOREWORD

The U.S. Environmental Protection Agency (EPA) is increasingly recognized as both a scientific and a regulatory agency. Yet the role of scientific research in developing effective regulatory policy and its contributions to informed decision-making are often obscured by the more easily understood social, economic, and political issues that tend to dominate the public debate. The fact remains, however, that it is the documented or suspected interrelationships among pollution sources, environmental concentrations, human exposures, and associated health effects that form the justification for policy decisions about safeguarding public health.

Research sponsored by EPA is frequently discussed and dissected along two sometimes conflicting dimensions—programmatic and scientific. Programmatic refers to the various regulatory programs that tend to view research through the prism of their statutory mandates and deadlines. The regulators primarily look to research to provide specific data that will be immediately relevant in meeting statutory deadlines. The scientific perspective, on the other hand, is more holistic and longer term. The scientists tend to view research as a way to elucidate underlying chemical, biological, or physical mechanisms that will eventually lead to a better understanding of key relationships—for example, the relationships between exposure and dose or between dose and effect. Scientists see their work as a series of incremental steps that will ultimately improve our ability to estimate true risk, while regulators view research as a tool to produce precise and timely answers to specific regulatory questions. Scientists divide their work along disciplinary (e.g., toxicology, epidemiology, clinical) or health outcome (e.g., genetic, reproductive, pulmonary, neurologic) lines, while the regulators see things along media (e.g., air, water, soil, food) or legislative (e.g., Clean Air Act, Toxic Substances Control Act) lines.

It is our intent in this document to lay out a strategic health research plan that is responsive to EPA's regulatory needs, but also consistent with the underlying scientific issues. A major goal is to show clearly that there is a common set of health research issues that cuts across media and regulatory programs. And furthermore, to make it clear that all the regulatory programs will benefit from a comprehensive and integrated health research program that addresses the major uncertainties in health risk assessment.

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Table of Contents

EXECUTIVE SUMMARY

In the past 20 years, major environmental legislation has given the U.S. Environmental Protection Agency (EPA) the regulatory tools it needs to protect our environment and public health. Environmental protection, however, requires more than legislative vehicles; appropriate regulatory decisions based on those laws must be founded on scientific data concerning the scope and magnitude of health risks associated with the environmental hazards to which the public is exposed. For most pollutants, that link between environmental exposures and adverse health effects is not yet clear. Moreover, the difficulty facing decision makers in weighing the known costs of pollution reduction or prevention against the ambiguous public and health benefit is becoming more acute in the face of severely constrained public resources.

EPA's health research program is well situated to address this informational gap through an integrated research strategy that assimilates and builds on related work in other federal agencies, as well as in the scientific community at large. Only at EPA is there a direct interface between the researchers, risk assessors, and risk managers for forging the most scientifically sound regulatory decisions. To function in this environment, EPA health scientists must be cognizant of important breakthroughs in the basic biologic sciences (e.g., genetics, molecular biology) and capable of applying these scientific advances to problems facing the Agency. Conversely, they must be knowledgeable about regulatory activities and able to recognize and conceptualize the basic research questions raised by contemporary environmental issues. In short, they function at the interface between basic and applied research. The Office of Health Research, and more specifically, the Health Effects Research Laboratory (HERL) is the focal point for these research efforts.

To address the broad range of environmental contaminants covered under various legislative statutes, HERL research must assist EPA in evaluating the health risks for diverse environmental agents. While the chemical and physical composition of these pollutants differs significantly, the evaluation of their health effects must address a common set of issues: exposure, or the extent to which humans are exposed to pollutants in the environment; dose, or the relationship between the exposure and the dose of the pollutant received at the site(s) of toxic action within the body; and effect, or the health impact from the pollutant dose. These fundamental issues form the risk assessment paradigm that underlies the research needs of all EPA regulatory program areas, and therefore they are central to the entire HERL program. These issues can be further subdivided into seven research topics that are addressed in the HERL program: exposure, dose-response, hazard identification, chemical-specific data, pollutant mixtures, and biological markers research as well as human data development.

HERL is functionally organized along the lines of the key scientific specialties in environmental health research. This document presents a strategic research plan for HERL that merges three key components: regulatory program concerns, key research topics, and HERL scientific divisions. The remainder of this report explains how these three components are synthesized in the HERL research strategy. In sum, the document explains the direction of EPA's health research program over the next five years, and the reasons for the prioritizations made by each division.

SECTION ONE
INTRODUCTION

1.1 OVERVIEW

The Health Effects Research Laboratory (HERL) within the Office of Health Research (OHR) is the focal point of the U.S. Environmental Protection Agency's (EPA's) effort to understand the human health impacts of environmental pollutants. The regulatory program offices within EPA use HERL's research results—either directly or after they have been incorporated by the Office of Health and Environmental Assessment (OHEA) into a risk assessment—to develop regulations that effectively and efficiently safeguard public health. HERL develops its research strategy and plans based on the needs of the regulatory programs and on the scientific uncertainties that must be resolved to fulfill those needs. HERL's scientific research is used, therefore, to protect public health from the effects of pollutants.

1.2 LEGISLATIVE MANDATE

EPA's and HERL's authority to conduct environmental health research is derived initially from the major federal laws mandating broad programs to protect public health and the environment. Each of these laws, including the Clean Air Act, the Safe Drinking Water Act, the Clean Water Act, the Toxic Substances Control Act (TSCA) the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Resource Conservation and Recovery Act (RCRA), and the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), requires that EPA develop regulatory programs to protect public health. Several regulatory programs established under each statute require specific consideration of health effects:

- **Air Quality** - National Ambient Air Quality Standards (NAAQS) are based on human health endpoints. State programs and federal emissions limits are designed to achieve those standards. Special emissions limits are established for pollutants that are hazardous to human health (i.e., toxic air pollutants). Radiation standards and radon guidance are also designed to protect human health. The health effects of pollutants generated by the combustion of alternative fuels and indoor air pollutants are emerging concerns.

- **Drinking Water** - National drinking water standards (Maximum Contaminant Levels, or MCLs) are based directly on human health endpoints. Health Advisories provide human health recommendations for some pollutants not covered under MCLs.

- **Water Quality** - EPA develops ambient pollutant limits (criteria) for surface waters based in part on human health endpoints. For permitting decisions, the states or EPA may require testing of effluents for toxicity. Regulations for sludge disposal are based on health risks.

- **Toxic Substances** - Health data collected on new and existing chemicals are used to determine whether to implement restrictions on the manufacture, use, and/or disposal of toxic chemicals.

- **Pesticides** - The pesticide registration, reregistration, and special review programs involve the evaluation of toxicity and other health-related information to assess the effects of pesticide products.

- **Hazardous and Nonhazardous Wastes** - Toxicity data are used to help determine which wastes are regulated as hazardous. Regulations for facilities that accept waste and restrictions on land disposal of waste are designed to protect the health of populations near disposal sites.

- **Superfund Waste Sites** - Emergency response and cleanup actions are designed to protect the health of human populations near waste sites.

Because each major environmental statute requires the development of health-protective programs, the statutes include specific legislative authority for EPA to conduct health effects research. The sections of each statute authorizing health research, and examples of environmental exposures covered under this research, are presented in Table 1-1. Based on these legislative authorities, HERL formulates its research strategy to provide scientific results that facilitate informed decision-making and rule-setting under each regulatory program.

Table 1-1:
MAJOR ENVIRONMENTAL LEGISLATION ADMINISTERED BY THE U.S. ENVIRONMENTAL PROTECTION AGENCY, SPECIFIC AUTHORIZATION FOR THE EPA TO CONDUCT HEALTH RESEARCH, AND EXAMPLES OF RELEVANT ENVIRONMENTAL EXPOSURES

Enabling Legislation	Authorizing Section	Language Authorizing EPA to Conduct Health Research	Examples of Relevant Environmental Exposures
Clean Air Act (CAA)	Title I. Part A., Sec. 103	The Administrator is given broad authority to conduct research relating to the "causes, effects, extent, prevention, and control of air pollution." Special emphasis should be given to research on the short- and long-term effects of air pollutants on public health and research to "improve our knowledge of the contribution of air pollutants to the occurrence of adverse effects on health, including, but not limited to, behavioral, physiological, toxicological, and biochemical effects."	■ National Ambient Air Quality Standard Pollutants (e.g., ozone, nitrogen, sulfur dioxide, carbon monoxide, inhalable particles, lead) ■ Hazardous Air Pollutants (e.g., benzene, formaldehyde, styrene)
	Part B, Sec. 153	The Administrator is given authority to conduct studies on "biomedical, or other research and monitoring...to ascertain any direct or indirect effects upon the public health and welfare of changes in the stratosphere, especially ozone."	
Safe Drinking Water Act (SDWA)	Part E, Sec. 1442	"The Administrator may conduct research...of physical and mental diseases and other impairments of man resulting directly or indirectly from contaminants in water, or to...improve methods to identify and measure the health effects of contaminants in drinking water."	■ Contaminated Drinking Water (e.g., lead, chlorinated solvents, trihalomethanes, pathogenic bacteria and viruses, natural organics)
Resource Conservation and Recovery Act (RCRA)	Subtitle H, Sec. 8001 (A)	The Administrator shall conduct...research, investigations, experiments...and studies relating to any adverse health and welfare effects of the release into the environment of material present in solid waste.	■ Hazardous and Municipal Wastes (e.g., contaminated soil, water, and air from hazardous, municipal, or medical wastes) ■ Solid Wastes (e.g., sewage sludge, incinerator ash) ■ Superfund Chemicals (e.g., contaminated soil, water, and air from Superfund sites, with emphasis on high priority chemicals listed in Section 313 of CERCLA)

Table 1-1: Continued

Enabling Legislation	Authorizing Section	Language Authorizing EPA to Conduct Health Research	Examples of Relevant Environmental Exposures
Superfund Amendments Reauthorization Act (SARA)	Sec. 209	The purposes of this section are to establish a comprehensive and coordinated Federal (SARA) program of research...to improve the scientific capability to assess, detect and evaluate the effects on and risks to human health from hazardous substances."	
	Sec. 403	"...The Administrator of the EPA shall establish a research program with respect to radon gas and indoor air quality... The research program required under this section shall include...research related to the effects of indoor air pollution and radon on human health..."	■ Indoor Air Pollutants (e.g., environmental tobacco smoke, emissions from building materials and unvented combustion appliances)
Clean Water Act (CWA)	Part 1254, Sec. 104	"...the Administrator shall conduct research on the harmful effects on the health and welfare of persons caused by pollutants in water..."	■ Contaminated Surface and Ground Water (e.g., industrial effluents, urban/rural runoff, municipal and hazardous wastes) ■ Contaminated Wetlands, Near Coastal Regions, and Oceans (e.g., sewage outfalls, industrial effluents, dumping of wastes, spills)
Toxic Substances Control Act (TSCA)	Sec. 10	"...research undertaken by the Administrator and directed toward the development of rapid, reliable, and economical screening techniques for carcinogenic, mutagenic, teratogenic, and ecological effects of chemical substances and mixtures."	■ Toxic Substances (e.g., manufactured/ processed substances such as PCBs, asbestos, solvents, etc., excluding pesticides)
Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)	Sec. 20	"The Administrator shall undertake research...to carry out the purposes of this Act..."	■ Pesticides (e.g., dinoseb, nitrofen, alar, captan, carbaryl)

1.3 RESEARCH TOPICS

To address the broad range of environmental contaminants covered under the statutes, HERL research must assist EPA in evaluating the health risks for diverse environmental agents including automotive and diesel exhaust, power plant emissions, pesticides and other toxic chemicals, hazardous waste, municipal solid waste, naturally occurring and genetically engineered microorganisms, drinking water disinfectants and associated by-products, and ionizing and nonionizing radiation. While the chemical and physical composition of these pollutants differs significantly, the evaluation of their health effects must address a common set of questions:

- **Exposure** - How and to what extent are humans exposed to the pollutant in the environment (i.e., route, magnitude, frequency, duration)?

- **Dose** - What is the relationship between this exposure and the dose of the pollutant received at the site(s) of toxic action within the body?

- **Effect** - What is the health impact of the pollutant dose?

These fundamental research questions underlie the research needs of all EPA regulatory program areas, and therefore are central to the entire HERL program. The questions can be further subdivided into seven research topics (four principal topics and three cross-cutting topics) that are addressed in the HERL program:

1. **Exposure Research** defines the route, magnitude, frequency, and duration of exposure of humans to environmental pollutants. Most exposure research efforts are carried out by another office within the Office of Research and Development (ORD), the Office of Modeling, Monitoring Systems, and Quality Assurance (OMMSQA), which is currently focusing its efforts on investigating the exposures experienced by individuals and populations during normal daily activities, with emphasis on identifying high-exposure groups. HERL efforts in this area focus on the development and validation of biological markers for exposure, effects, and susceptibility in human populations; these efforts involve substantial interaction with ORD monitoring laboratories.

2. **Hazard Identification Research** develops, refines, and validates approaches and methods for identifying potential human health hazards. HERL is developing techniques to determine causal relationships between environmental pollutants and adverse health outcomes that are faster, more accurate, less expensive, and more reliable than current options. This research includes the development of test methods for screening and characterizing new and existing chemicals and procedures to evaluate qualitative and quantitative relationships between the chemical structure of pollutants and their related biological effects (structure-activity relationships [SARs]).

3. **Dose-Response Research** elucidates a) the relationship between exposure (i.e., applied dose), dose at the site of toxic action (i.e., target dose), and biological effects; and b) the basic biological mechanisms responsible for the observed effects. HERL's research in this area includes developing better methods to relate exposure to dose (i.e., physiologically based pharmacokinetic models) and exploring the physiological and biological mechanisms of toxicity, including compensatory processes, that are crucial to accurate extrapolation of research results (e.g., extrapolating results from animals to humans, from high to low dose, and from acute to chronic effects).

4. **Chemical-Specific Research** develops scientific data on individual pollutants at the request of the regulatory program offices. HERL's efforts in this area include the evaluation of methods for screening and characterizing agents for which critical data gaps have been identified, generation of chemical-specific dose-response data using laboratory animals, and human clinical studies to investigate the acute effects of specific air pollutants. This research is generally short-term (1-2 years) and aimed at filling data gaps concerning specific chemicals or chemical mixtures that are of immediate importance to a regulatory program.

In addition to these four principal research topics, three other research themes that cut across the four main areas are of sufficient importance to be considered major research topics within the HERL program.

5. **Pollutant Mixtures Research** clarifies the extent to which synergistic, antagonistic, or additive interactions cause the effects of exposure to a mixture of pollutants to differ from the effect that would be predicted based on the characteristics of the individual pollutant components. HERL efforts will focus increasingly in this area because most common human exposures to environmental contaminants (e.g., urban air pollution, drinking water, incinerator emissions) involve pollutant mixtures.

6. **Biological Marker Research** develops and validates biological measurements that can be used to calculate dose at the site of toxic action and to detect effects at cellular and molecular levels. HERL will focus on the development of biological markers in humans for exposure, effects, and susceptibility. The use of these techniques in epidemiologic investigations will facilitate more direct assessment of exposure and effects, and thereby reduce related uncertainties.

7. **Human Data Development** consists of the collection of information on exposure, dose, and effects in hu-

man populations. Scientists at HERL and elsewhere in EPA develop and use human data to assess the status of public health, to identify potential environmental health problems, and to evaluate the efficacy of risk reduction measures. Human data are used also to identify and evaluate subgroups that are at higher risk because of increased susceptibility or elevated exposures, and to ascertain the degree to which effects observed in animals are analogous (or homologous) to those observed in humans. Research activities in this area include epidemiological and clinical studies and the establishment and maintenance of exposure and disease registries.

The health effects concerns of all EPA's regulatory program offices fall under these seven research topics. The Office of Health Research recently undertook a survey of the regulatory program offices to determine the priorities they assign to these topics. The results are presented in Table 1-2. The rankings by a given regulatory program office represent the relative, not absolute priorities of that office. Therefore, the assignment of a relatively low priority for a given topic does not necessarily mean that the topic is not important to the program office, but only that the topic has a lower priority than the other research areas.

The data presented in Table 1-2 reinforce the fundamental and essential nature of the seven research topics across diverse regulatory programs. All seven regulatory program offices rated dose-response assessment as a high priority, while five rated the collection of human data a high priority. Chemical-specific information and pollutant mixtures were a high priority for four programs, hazard identification and exposure assessment for three, and biological markers for two.

Table 1-2:
SUMMARY OF RESEARCH PRIORITIES BY REGULATORY PROGRAM

	Regulatory Programs						
Topics	**Air**	**Drinking Water**	**Water Quality**	**Toxics**	**Pesticides**	**Hazardous Waste**	**Superfund**
Hazard Identification	xx	x	xxx	xxx	xxx	xx	x
Dose-Response Assessment	xxx	xxx	xxx	xxx	xxx	xxx	xxx
Exposure Assessment	xxx	x	xx	xx	xx	xxx	xxx
Chemical-Specific Information	xxx	xxx	x	x	x	xxx	xxx
Pollutant Mixtures	xx	xxx	xxx	x	xx	xxx	xxx
Biological Markers	x	x	x	xx	x	xxx	xxx
Human Data	xxx	xxx	x	x	xxx	xxx	xxx

x = low priority xx = medium priority xxx = high priority

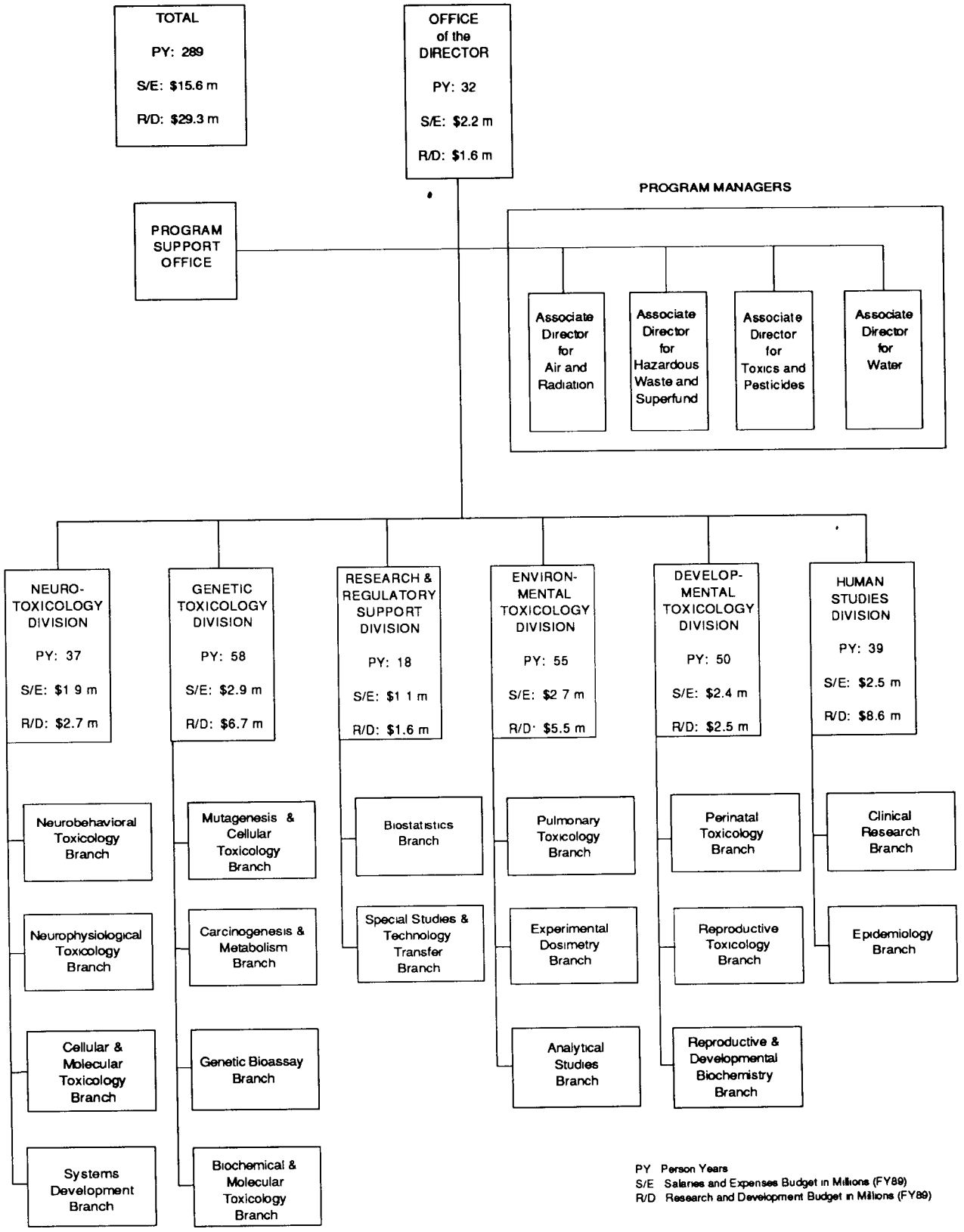

Fig. 1 - 1: Health Effects Research Laboratory

1.4 ORGANIZATION OF HERL

To effectively carry out its mission to provide health research to support EPA's regulatory programs, HERL is organized in accordance with the key scientific specialties in the environmental health field (Figure 1-1). The functional role of each of the divisions is summarized as follows:

1.4.1 Office of the Director

The Office of the HERL Director includes the Director and Deputy Director, as well as four Associate Laboratory Directors (Program Managers), who ensure that the scientific program is responsive to Agency needs; and the Program Support Staff, who are responsible for budgetary and administrative oversight. In general, this office oversees and coordinates the activities of the laboratory, including interactions with other parts of EPA and outside organizations.

1.4.2 Neurotoxicology Division

The Neurotoxicology Division plans, conducts, supports, and evaluates research to examine the effects of physical and chemical agents on nervous system function. In the course of examining toxicant-induced changes in the developing and adult nervous systems, the program touches on all levels of neural organization, including functional and structural. Behavioral evaluations include measurements of sensory, motor, and cognitive integrity. Extensive efforts are underway to develop methods to screen chemicals for neurotoxic potential and to develop detailed information regarding the specificity of effects of toxic chemicals.

Neurochemical research encompasses measurements of exogenous chemicals in biological tissues and studies of the influence of neurotoxicant exposure on endogenous chemicals (e.g., neurotransmitters and their associated synthetic and degradative enzymes, neurotoxic esterase, nervous system specific proteins) and chemical processes (e.g., energy metabolism, axonal transport). The neurophysiology program focuses on direct measurements of nervous system activity, including evoked potentials, EEGs, and seizure susceptibility. Measurements are generally made *in vivo* and are correlated with behavioral, neuropathological, and biochemical indicators of neurotoxicity.

1.4.3 Genetic Toxicology Division

The research program of the Genetic Toxicology Division improves EPA's understanding of environmentally induced mutagenesis and carcinogenesis, with special emphasis on pollutant mixtures and biological markers of exposure and effects (e.g., biochemical, molecular, and cellular indicators). The program encompasses research on the metabolic activation and detoxification of environmental pollutants that act through genetic mechanisms, as well as studies on alternative mechanisms for mutagenesis and carcinogenesis. The division has the capability to evaluate the mutagenic and oncogenic potential of environmental agents, both singly and in combination, through stepwise application of structure-activity analysis, short-term screening tests, and confirmatory bioassays for mutagenesis and carcinogenesis using laboratory animals.

The objective of mutagenesis research is to understand the chemical induction of somatic and germ cell mutations as a basis for improving risk assessments for cancer, reproductive failure, and heritable mutations. The carcinogenesis component of the research program is aimed at achieving a better understanding of chemical carcinogenesis in order to improve cancer risk assessments. Laboratory research is undertaken to examine the ability of chemicals to influence the induction, promotion, and progression of cancer; while theoretical studies are carried out to relate the molecular structure of chemicals to potential biologic effects. Research on pollutant mixtures is designed to develop, validate, and refine bioanalytical methods to qualify and quantify human exposure, molecular dose, and associated genotoxic effects. The division has the capacity to apply genetic bioassays in the field to detect, identify, and compare potential human health hazards from exposures to mixtures of pollutants.

Substantial efforts are also aimed at identifying and validating biological markers for genotoxicants that can provide quantitative data on exposure and dose, and that can produce meaningful information about the relationships among early biochemical changes, preneoplastic lesions, and cancerous lesions. This work will further the field of molecular epidemiology and will aid in the development of biologically based dose-response models for mutagenicity and carcinogenicity.

A relatively new area is research concerning the environmental release of naturally occurring and genetically engineered products from the biotechnology industry. Currently, there is a small program to identify the potential human health hazards that might arise from this tech-

nology, with initial emphasis on the development of suitable screening tests.

1.4.4 Environmental Toxicology Division

The Environmental Toxicology Division conducts both *in vivo* and *in vitro* animal research to determine the potential for human health risks from a broad spectrum of environmental pollutants involving different routes of exposure. This research is designed to determine cause and effect relationships at pollutant concentrations and exposure patterns that mimic those occurring in the environment. Studies focus on the pulmonary and cardiovascular systems, the immune system, the skin, and the liver. Although much of the research involves inhalation toxicology, exposure of animals to toxic substances by other routes and the development of route-to-route extrapolation methods are also important components of the research effort. These efforts reflect the division's commitment to develop physiological models for describing the pharmacokinetic behavior of environmental chemicals. Experimental studies to develop the database to predict high-to-low dose, acute-to-chronic, and interspecies extrapolations are being conducted that form the basis for dosimetry models. In addition, the division emphasizes studies to determine absorption, distribution, metabolism, and elimination of toxicants.

1.4.5 Developmental Toxicology Division

The Developmental Toxicology Division conducts biological research to detect, interpret, and extrapolate the effects of environmental pollutants, singly or in combination, on reproduction and development. Major emphasis is on the development of new and improved methodologies for the assessment of embryo and fetal toxicity, postnatal functional deficits, and male and female reproductive toxicity. Studies focus on the morphological, biochemical, and physiological assessment of germ cell function, gonadal function, and embryonic development in both the normal and abnormal situation. The environmental agents currently under investigation include toxic substances, chemical and microbial pesticides, air pollutants, drinking water contaminants, and hazardous wastes.

The perinatal toxicology research conducted by a branch of this division examines the potential developmental toxicity when environmental exposure occurs between fertilization and sexual maturation. The reproductive toxicology research conducted by the division is aimed at defining the effects of environmental agents on the functional integrity of the reproductive system, and determining the significance of data generated in animal studies to the human situation.

The reproductive and developmental biochemistry research focuses particular attention on additives and contaminants found in drinking water. Efforts are directed toward understanding the relationship between maternal and developmental toxicity by varying exposure scenarios, examining the consequences of changes in maternal homeostasis and body burden during different developmental stages, developing and validating novel techniques for evaluating male reproductive health, and comparing the results of *in vivo* and *in vitro* bioassays for developmental effects.

1.4.6 Human Studies Division

The Human Studies Division conducts both clinical and epidemiological investigations to improve the understanding of human health risks associated with environmental pollution. Clinical studies are conducted on research questions that are best approached under highly controlled laboratory conditions, whereas epidemiologic investigations rely on field studies in more natural settings. The goal of many research projects is to better characterize the similarities and differences between effects observed in humans and those in animals, or between results of *in vivo* and *in vitro* tests.

The Clinical Research Branch emphasizes controlled laboratory experiments to study primarily the health effects of inhaled air pollutants in humans. The research emphasizes several different, but complementary, measurements: the deposition, fate, and biologic effects of inhaled gases and particles; pulmonary and cardiovascular function; neurobehavioral function; pulmonary and systemic immunity and host defenses. Study populations are volunteers and include healthy adults, the elderly, children, and patients (e.g., those with respiratory infections, asthma, chronic obstructive lung disease, or ischemic heart disease).

The Epidemiology Branch conducts research that involves the collection of human data through field studies or the analysis of existing databases. The research is focused on three major areas: 1) airborne pollutant exposures (e.g., photochemical oxidants, hazardous air pollutants) and their effects on human health; 2) the human health effects of exposures to pollutants in drinking water, wastewater, and sewage; and 3) the human health effects of exposures to hazardous substances (e.g., pesticides, toxic substances, and hazardous waste materials).

1.4.7 Research and Regulatory Support Division

This division has the following responsibilities: coordinating multimedia, multidivisional projects; examining current and future trends that are likely to affect the direction of environmental health research; synthesizing, summarizing, and reviewing health effects information, especially as it relates to the Agency's regulatory responsibilities; and acting as liaison to the regional offices, state and local agencies, and the public. The division also provides statistical and mathematical support to all components of the laboratory.

1.5 STRUCTURE OF THIS REPORT

The previous discussion described the broad health effects concerns of EPA's regulatory programs, identified seven basic health research topics that encompass all of these concerns, and described the functional organization of HERL along the lines of key scientific specialties in environmental health research. HERL's research strategy merges these three components: regulatory program concerns, key research topics, and HERL scientific disciplines. The remainder of this report explains how these three components are synthesized in the HERL research strategy.

Figure 1-2 depicts the three components of the HERL strategy as a three-dimensional matrix, with the research topics serving as a link between the regulatory program offices and the HERL research disciplines. This linkage can be explained as follows: the environmental health concerns of the regulatory programs all fall within the seven research topics; the research questions of the scientific disciplines of HERL also fall within the seven research topics. Thus, any cell in the matrix represents a research topic that is both a concern of one of the EPA regulatory programs and addressed by HERL.

The remainder of this document explains this linkage in more detail. Section Two explains how each research division of HERL establishes its priorities and selects research projects within the seven research topics. Section Three explains how the concerns of EPA's regulatory programs under each research topic are addressed by the HERL program.

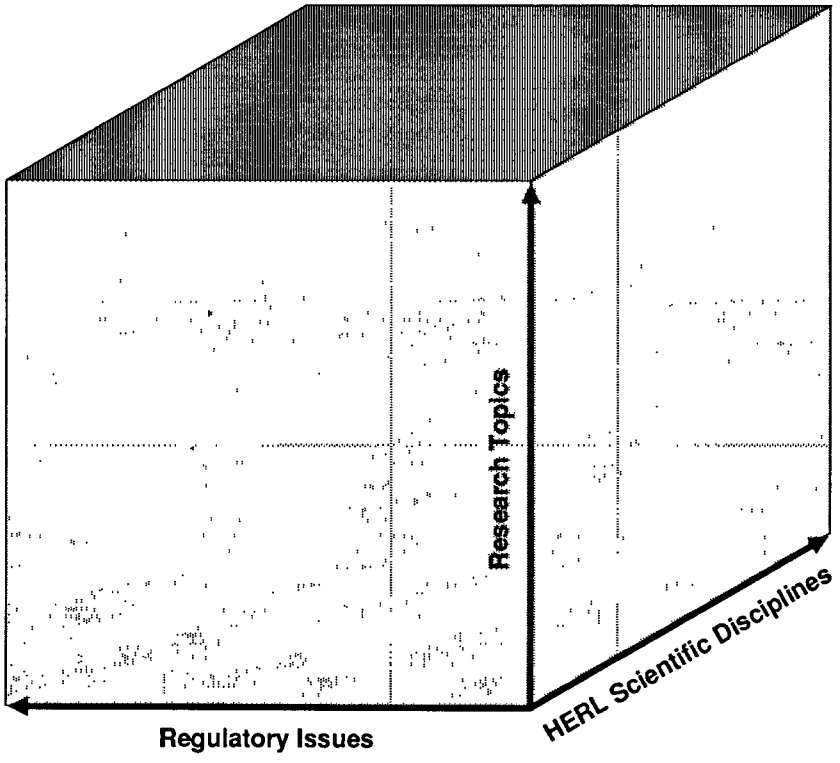

Fig. 1-2: Dimensions of OHR Research Program

SECTION TWO
CURRENT AND FUTURE RESEARCH DIRECTIONS BY DIVISION AT THE HEALTH EFFECTS RESEARCH LABORATORY

In this section, current and future research efforts under the EPA health program are separated first by scientific specialty and then by research topic (the z and y axes in Figure 1-2). Section 2.1 provides an overview of the HERL research program, outlines specific research needs of program offices that must be met by the research program, and introduces HERL efforts in the scientific disciplines relevant to these needs. Sections 2.2-2.6 detail the work performed by the different HERL divisions (neurotoxicology, genetic toxicology, environmental toxicology, developmental toxicology, and human studies). This discussion presents the scientific point of view on the Agency's current and future health research needs.

2.1 OVERVIEW: HEALTH EFFECTS RESEARCH LABORATORY

2.1.1 HERL Research Program

OHR/HERL is responsible for supporting the Agency's regulatory programs through a strong research program in environmental health. The regulatory programs require research to improve the accuracy of environmental health risk assessment. Addressing the needs of various program offices involves projects that span the gamut from short-term, applied research to more long-range, basic research.

To run such a health program, EPA scientists must have expertise in conducting research at all levels of function (i.e., molecular, intracellular, cellular, tissue, organ, whole organism). They must be aware of important breakthroughs in the basic biological sciences (e.g., genetics, molecular biology) and at the same time be capable of applying these scientific advances to problems facing the Agency. Conversely, they must be knowledgeable about regulatory activities and able to recognize and conceptualize the basic research questions raised by contemporary environmental issues. In short, they function primarily at the interface between basic and applied research, ensuring rapid and productive communication between these two ends of the research spectrum (see Figure 2-1).

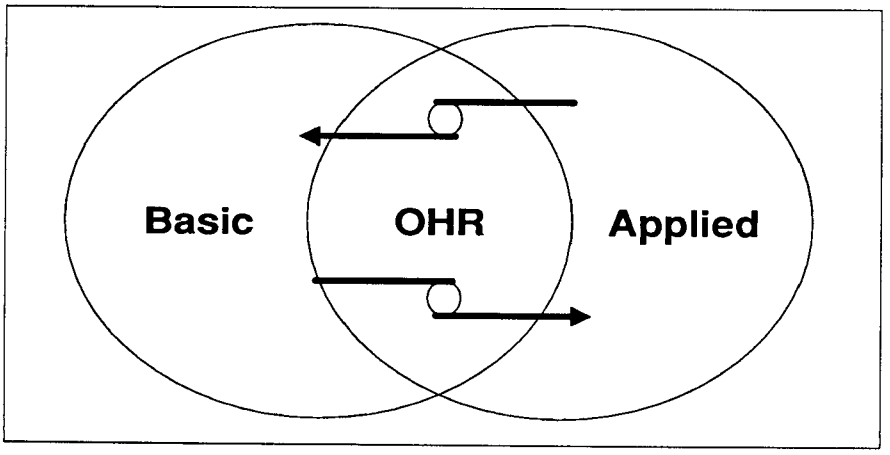

Fig. 2-1: The Interface Role of EPA Health Scientists

HERL uses the risk assessment paradigm outlined in Section 1 to structure a health research program that addresses the regulatory needs of the program offices. Assessing the risk associated with exposure to a particular pollutant requires defining and characterizing the factors that influence both the movement of the pollutant from source to dose levels in target organ(s), and the response of those organ(s) to the pollutant dose. (Important examples of these factors are listed in Figure 2-2.) HERL's analysis of these factors will enhance con-

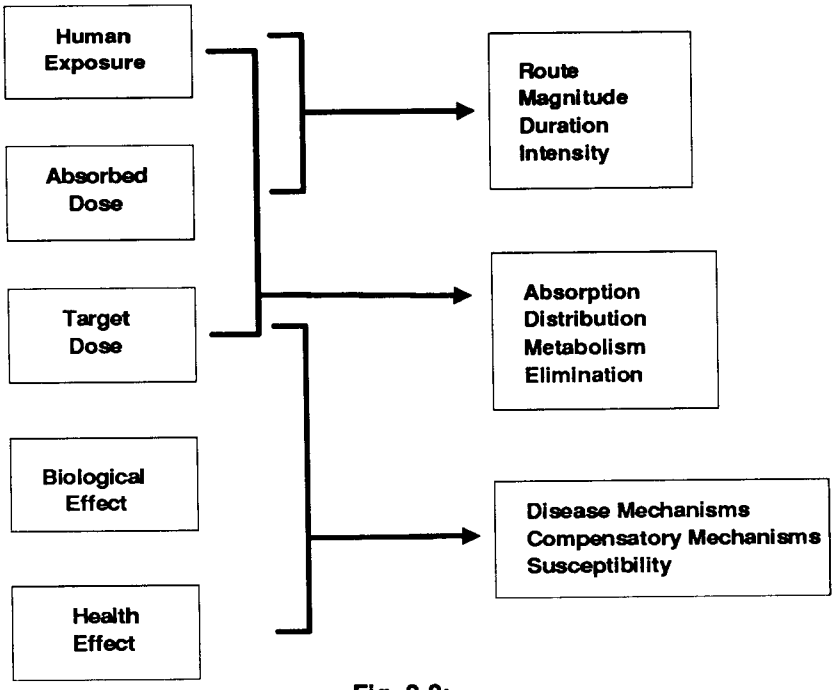

Fig. 2-2:
Factors Influencing Pollutant Movement and Dose-Response

The products of the research program include:

- State-of-the-science **methods** for use by the Agency in hazard identification and the biologic (dose) aspects of exposure assessment
- **Models** for extrapolation of dose and effect needed for dose-response assessment
- Application of methods and models to collect scientific data (**measurements**) on specific pollutants requested by the regulatory programs

fidence in its interpretation of data obtained directly from human populations and will also lessen the uncertainty of extrapolating risk to human populations from data obtained from laboratory animals. The manner in which research to understand the unknown events between exposure and health effects supports the different components of the risk assessment process is illustrated in Figure 2-3.

Within the risk assessment paradigm, the health research program focuses on hazard identification and dose-response assessment, which are the steps directly concerned with the health consequences of human exposure to pollutants. Research to improve hazard identification primarily involves efforts to develop methods for screening (detection) and characterization of health hazards (e.g., fate of agent within the body; identification of putative targets) and provides presumptive evidence of causality between exposure and effects. Research to improve dose-response assessment primarily involves efforts to develop predictive models of dose (i.e., physiologically based pharmacokinetic models) and effects (i.e., biologically based dose-response models) that allow quantitative integration and extrapolation of dose and effect. Support is also provided for the facets of exposure assessment that rely on measures of applied and delivered dose (e.g., body burden, biomarkers) in constructing more precise models of actual human exposure.

2.1.2 Laboratory-Specific Research Needs

The various program offices in EPA give different priority ratings to all the research topics outlined in Section One except for dose-response assessment (see Table 1-2); the high-priority rating given this topic by all the offices reflects a universal need for predictive models of dose and effect. Within a particular discipline, the overall balance of the research program at any point in time is determined both by the program office needs and by the state-of-the-science in that discipline. Often, fundamental research is required to provide the framework by which an issue of concern can be directly addressed.

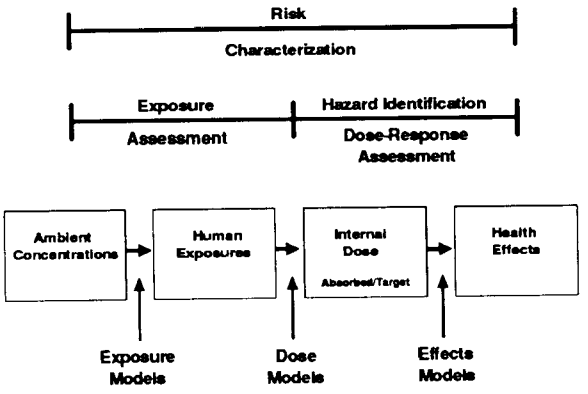

Fig. 2-3: HERL Support of Risk Assessment Needs

For example, sensitive and reliable measures to characterize health effects (i.e, hazard identification) are a prerequisite for developing predictive models. These methods have been developed for some target systems and processes (e.g., genotoxicity), but not for others (e.g., immunotoxicity). Thus, in some areas of health research (e.g., developmental neurotoxicology), research efforts in hazard identification must precede work on predictive models"even though the development of such models is the predominant goal of health research.

Following is a brief discussion of research needs under the two topics of hazard identification and dose-response assessment. These needs are addressed by all divisions of HERL.

2.1.2.1 Hazard Identification

If no toxicologic data are available for a chemical, investigators must first focus their efforts on answering this question: What health effects are produced by exposure to the chemical? Answering the question involves developing and/or running procedures that screen for possible effects. Once researchers suspect a chemical produces a particular type of health effect, efforts can turn to characterizing the nature and defining the mechanism for these effects. This research may pinpoint the target organ(s) responsible for the effect(s), improve the sensitivity of the quantitative dose-effect estimations, and assess the similarity in expected response between the laboratory species and humans (if the data were not obtained from humans).

Hazard identification research has two primary goals: 1) to develop, refine, and validate methods for use in screening and characterizing potential health hazard; and 2) to apply these methods to the characterization of specific chemicals of interest to the regulatory programs.

Screening. HERL needs methods to evaluate the potential for new and existing chemicals to cause potential health hazards. These methods should be rapid, simple, and economical to administer, while providing reliable qualitative, presumptive evidence for a particular type of health effect (e.g., genetic damage). Screening methods have two general applications: 1) to determine if exposure to a specific chemical can produce a particular health effect and whether further testing is required, or 2) to establish which of the large number of chemicals now released into the environment should be tested first.

The development of screening methods to prioritize chemicals for further testing is a prime example of a risk assessment-related activity that benefits the program offices but has limited applicability to setting regulatory levels. Research in this area, therefore, is not a major component of the health research program. Given the increased Agency concern for noncancer health effects, however, the development of *in vivo* methods that focus on some of the newer areas of health concern (e.g., immunotoxicology) will be supported. Similarly, the development of *in vitro* approaches to screening, especially in areas in which short-term, cost-effective tests have not been available (e.g., neurotoxicology), will receive increased attention. These *in vitro* models can also be used by researchers examining cross-species mechanisms and pharmacokinetics"both important components in developing predictive models of dose and effect.

HERL also needs to concentrate on developing qualitative and quantitative relationships between chemical structure and related biological activity (i.e., structure-activity relationships [SARs]). A better understanding of SARs will aid in identifying/predicting health effects associated with new chemicals and setting priorities for toxicity testing. Before SAR research can be more fully developed, however, HERL needs better methods for identifying effects and better approaches for evaluating chemical structure.

Characterization. Developing methods to **characterize hazard** is a much more demanding task than developing screening methods because the former are used to precisely define and verify relationships between exposure and dose and between exposure and various health effects. HERL needs approaches for specifying the target(s) of toxicity, the progression of toxicity with dose and time, the severity of given effects, and the interaction of concurrent or sequential toxicities. Furthermore, for a given target organ, researchers may need a number of methods to characterize the variety of possible health outcomes and their inter-relationships. These methods must be sufficiently rigor-ous to associate single or multiple mechanisms/targets with the expression of single or multiple health effects. For example, as a result of either single or multiple mech-anisms, a neurotoxicant may produce a variety of outcomes (e.g., learning disabilities, visual impairment, motor dysfunction).

The use of sensitive test methods to characterize health outcomes can also serve as a major source of input data for predictive models. Thus, because of the importance placed on these models, research related to hazard

characterization (as opposed to screening) is a major component of the health research program.

HERL also needs methods to **characterize the dose of a chemical to a target organ.** The uptake, distribution, and disposition of a chemical within the body is influenced by the concentration (dose) to which a person is exposed, the duration of that exposure, and the portal of entry for the pollutant. Analyzing these influences allows better estimation of health effects in the various body systems. For example, the pulmonary function may be impaired by lower levels of an inhaled pollutant than the reproductive function"not because the lungs are more sensitive to the chemical than the reproductive tract, but because more of the chemical contacts the lungs (the portal of entry). The growth of a pharmacokinetic program in HERL will foster research efforts to develop these methods for characterizing target dose.

2.1.2.2 Dose-Response Assessment

A dose-response assessment estimates the relationship between the magnitude of the exposure to a pollutant and the probability that the health effect in question will occur. Often the data needed to make this assessment are not available, and risk assessors must instead estimate the relationship by extrapolating from data gathered under different conditions. Most exposures to humans involve low doses and chronic exposures, while most available data on various chemicals have been gathered on laboratory animals, at high doses and with acute exposures. Alternatively, either animal or human data may be available for a given chemical, but the information was gathered in relation to a different route of exposure than that under current examination.

Because of the paucity of appropriate available data, and because of a lack of understanding of the underlying biological mechanisms responsible for health effects, large uncertainties hamper the risk assessment process. HERL needs to develop, refine, and validate models which predict the dose of a chemical that reaches the human target tissue as well as the health effects that will result. To be accurate, these models must take the underlying biological processes that are affected by chemical exposure into account. Two types of models are needed:

- **Predictive Models of Dose.** Over the last few years, a goal of quantitative risk assessment has been to use "delivered dose""the dose of proximate toxicants, whether parent compound or metabolite, at the tissue site of toxic action"rather than applied dose or ambient concentration. Determining this delivered dose is an extension of exposure assessment, in that the direct exposure of the target tissue is examined free of the various physiological fate and transport processes by which the body filters, attenuates, degrades, and modifies compounds absorbed from its environment. To reduce uncertainty in the risk assessment process, however, scientists must analyze the effects of different conditions of exposure on the amount and pattern of delivered dose. For example, being able to define the delivered dose of a pollutant would be very useful in comparing the toxic effects of exposures to that pollutant by different routes of administration. In this way, extraneous factors such as different degrees or rates of absorption can be accounted for, resulting in more meaningful comparisons. Research to determine ways to estimate tissue-level doses is also necessary for progress in mechanistic biological modeling of toxicity, which requires extensions of exposure assessment to the internal sites where these mechanisms occur.

  These data are critical to the ultimate focus of this effort: the development of theoretical and computational models to predict dose levels, which can then be compared across species and across exposure scenarios. In the past, researchers used compartmental models that reflected primarily mathematical constructs. The proposed research in this area will generate data for pharmacokinetic models that use as input physiological parameters of the system being modeled as well as the molecular structures and reactivities of the pollutants under consideration. Validation of these models will allow researchers to scale exposure, dose, and effects data observed in one situation to different exposure conditions and/or populations.

- **Predictive Models of Effects.** The ultimate goal of dose-response assessment is to estimate the incidence of a particular health effect at human exposure levels. Extension of the dose-effect curve downward from the levels at which laboratory animals are exposed to the levels at which humans are usually exposed requires an understanding of the various rates of attack, repair, and propagation of damage across species. HERL, therefore, needs to develop biologically plausible dose-response models that consider the potential for different biological mechanisms to elicit, initiate, or contribute to the health effect of concern. The primary focus of this research is to better understand the role of various biological processes on chemically induced injury and to produce models

flexible enough to incorporate new information as it is obtained.

Specific research areas include 1) intra- and inter-species extrapolation (e.g., mechanisms of action, species and subpopulation sensitivity); 2) extrapolation of health effects across different exposure scenarios; and 3) incorporation of recent mechanistic concepts into predictive models.

2.1.3 Research Plan

The health research program is organized to address the major categories of health effects that result from exposure to environmental chemicals, including genetic, developmental/reproductive, neurobiologic, pulmonary, and immune effects. Limited programs also exist to evaluate cardiovascular and hepatic effects. Recently, HERL has also developed a formal program to evaluate pharmacokinetics. In each scientific discipline, HERL prioritizes research by integrating Agency needs (mission) with scientific priorities dictated by the state-of-the-science in that area. The following discussion introduces the laboratory priorities and directives for each of these disciplinary areas in relation to the two most important research topics for determining health effects"hazard identification and dose-response assessment. The divisions apply these methods and models to chemicals of regulatory interest; information gathered in these efforts falls under the research topic chemical-specific data. Finally, the divisions examine some research topics that cut across hazard identification and dose-response assessment, such as biomarkers. Note that in the more detailed discussions of the divisions' activities in the remainder of the section, cross-cutting research efforts are outlined under separate sections; however, in the summaries of each division's activities, the cross-cutting efforts are included under hazard identification and dose-response (as appropriate).

2.1.3.1 Neurotoxicology

The neurotoxicology program is designed to identify, quantify, and characterize the effects of environmental pollutants on the adult and developing nervous system. In order to evaluate these effects thoroughly, research efforts encompass all levels of neural organization, including whole animal, cellular, subcellular, and molecular.

Current interest in the use of noncancer endpoints for risk assessment has focused considerable attention on neurotoxicology. Various regulatory programs in EPA are planning to require recently developed *in vivo* screening tests to be used in routine testing. There is a clear need, however, for *in vitro* tests to complement or replace the more costly *in vivo* tests. The Agency is committed to support research using *in vitro* technology and thus will place an increased emphasis on these needs in neurotoxicology.

A distinctive characteristic of the nervous system is the complexity of the processes it mediates and controls. Perhaps unlike any other area of health research, neurotoxicology requires the development of a variety of validated test methods that can be used to identify, quantify, and characterize neurotoxic outcome following toxicant exposure. Researchers will continue to develop and validate test methods, especially those for evaluating sensory, motor, and cognitive dysfunction produced by environmental chemicals. Research in this program will determine the extent to which current assessment procedures can predict the appropriate neurotoxic outcomes in humans. Studies on interactive effects of various classes of neurotoxicants will also be conducted.

Neurotoxicity can be produced by a variety of mechanisms that can be studied at several levels of neuronal organization. Research, therefore, will focus on understanding mechanisms of neurotoxicity and factors that affect neurotoxic outcome (e.g., compensatory mechanisms, exposure conditions). A related issue is that neurotoxic outcome is dependent on the extent and site of insult. Research will focus on understanding the relationships between neurotoxicity measured at the various levels of neuronal organization.

A first step in the development of predictive models for neurotoxicity is a better understanding of extrapolation issues related to age, species, route, and exposure scenario. Research efforts will thus address the ability of different test methods to predict effects across species and as a function of various dosing scenarios and routes of administration. Included in the area of species extrapolation is the development of methods that provide a link between what can be measured in humans and homologous animal models. HERL also needs data concerning adaptation, recovery of function, and developmental and delayed neurotoxicity.

This research represents both new areas of emphasis and growth areas for the existing program. Efforts to validate methods for characterizing neurotoxic outcome and for extrapolating these results to humans reflect a general evolution in the state of the science. A major new

area of research for the neurotoxicology program will be *in vitro* test methods. Future research directions in neurotoxicology can be highlighted as follows:

- *Hazard Identification*
  - Complete validation of primary *in vivo* screen for routine neurotoxicological assessments
  - Develop and validate *in vitro* tests to be used in routine neurotoxicological assessments
  - Validate methods to characterize neurotoxic outcome, linking effects observed at the screening level to effects quantified at the secondary levels
  - Develop and validate methods and approaches to identify and characterize developmental neurotoxicity
  - Develop and validate cellular and molecular predictors for neurotoxicity
  - Evaluate structure-activity relationships of neurotoxic agents using *in vivo* or *in vitro* procedures
- *Dose-Response Assessment*
  - Evaluate cellular and molecular mechanisms of neurotoxicity
  - Emphasize studies concerning extrapolation issues, such as route-to-route, dosing scenario, species, age, stress, and high-to-low-dose
  - Understand mechanisms of compensation, recovery of function, and delayed and/or long-term dysfunction produced by acute or intermittent exposures
  - Address the additivity hypothesis for combinations of neurotoxicants on high-to-low-dose extrapolation

2.1.3.2 Genetic Toxicology

The genetic toxicology program is designed to explore the influences of environmental pollutants on genetic changes in somatic and germinal tissues (mutagenesis) and the conversion of normal cells to neoplastic cells (carcinogenesis). Researchers analyze both direct and indirect interactions with genetic material for environmental chemicals, complex mixtures, and genetically engineered microorganisms.

Historically, hazard identifications and risk assessments in the Agency have emphasized cancer as a health endpoint. Considerable progress has been made in developing both *in vitro* and *in vivo* methods for detecting genotoxic chemicals and in understanding the relationships between the genotoxicity and the carcinogenicity of environmental chemicals. Within the framework of the risk assessment paradigm, research emphasis is now shifting toward the development of data that will allow the extrapolation of results between *in vitro* and *in vivo* systems and between rodent and human systems. In doing so, researchers will also shift emphasis to *in vitro* and *in vivo* human systems.

The induction of cancer involves many potential mechanisms (e.g., gene or chromosomal mutation, heritable changes in DNA transcription reflected in altered gene expression) and multiple stages in tumorigenesis. Researchers will therefore focus on understanding mechanisms of carcinogenesis and the factors that modulate neoplastic changes. Because certain substances seem to induce tumors without appearing to induce genotoxic effects, HERL must also develop methods and models for nongenotoxic carcinogens.

The development of predictive models will be facilitated by the development of biomarkers for exposure and effects. The division will continue to emphasize the application of DNA adduct dosimetry in exposure assessment for genotoxic chemicals. Efforts will also be directed to understanding the relationship between molecular markers (e.g., oncogenes and tumor suppressor genes) and mechanisms of cancer development.

These research areas reflect a shift in emphasis from the current genetic toxicology program. Less emphasis will be placed on the application of short-term bioassays and greater emphasis on understanding the issues related to extrapolation that are crucial to dose-response modeling and risk assessment. Future research directions in genetic toxicology can be highlighted as follows:

- *Hazard Identification*
  - Develop computational tools to predict the toxicological activity of chemicals based on molecular structure and existing data

- Develop *in situ* genetic tests (plants and animals) for environmental monitoring and personal monitoring methods to quantify human exposure to genotoxic agents

- Improve gene cell assays for heritable genetic damage

- Improve methods to detect and characterize the effects of nongenotoxic carcinogens

- Improve approaches to identifying genotoxic components in complex mixtures, including interpretation of data, but decrease emphasis on actual application (i.e., sample collection and chemical characterization)

- *Dose-Response Assessment*

  - Examine molecular mechanisms of cancer (both for genotoxic and nongenotoxic chemicals)

  - Explore the molecular basis of somatic and gene cell mutation

  - Develop models to extrapolate between *in vitro* and *in vivo* systems and between rodents and human systems

- *Cross-Cutting Issues*

  - Develop biomarkers of exposure and effect with emphasis on molecular dosimetry and molecular alterations

2.1.3.3 Pulmonary Toxicology

The pulmonary toxicology program, which is jointly directed by the Environmental Toxicology Division and the Human Studies Division, is designed to explore the effects of environmental pollutants on pulmonary function in both laboratory animals and humans. The program's major research components in both laboratory work with animals and clinical and epidemiologic studies with humans make it unique in HERL. The program emphasizes examination of the effects of inhaled pollutants, including both gases and particles.

Historically, pulmonary effects"most notably, of criteria pollutants"have played a prominent role in the Agency's regulatory program in air. A variety of pulmonary function tests are available for hazard identification in both laboratory animals and humans. Human clinical studies, however, are limited to examining acute exposures at or near ambient concentrations. Consequently, major uncertainties still exist about the development of chronic lung disease following long-term exposure to air pollutants and the extent to which animal models of lung disease predict the human response. A continuing major focus of the pulmonary research program is the extrapolation issues related to mechanisms of lung injury across species and across exposure conditions.

The development of predictive animal models will be facilitated by parallel studies in rodents, nonhuman primates, and humans focusing on similar endpoints with relevance to mechanisms of lung injury and host defenses. These studies will be extended to address the relationship between lung dosimetry and pulmonary effects. Mechanistic studies in animals will also continue to focus on the pathogenesis of toxicant-induced lung diseases. The development of animal models for pulmonary disease (e.g., emphysema, asthma, obstructive and restrictive lung disease) will permit parallel studies with human subjects to address the issue of susceptible populations. Research to define biochemical markers that reflect early changes in lung function (e.g., biomarkers of pulmonary immune function and fibrotic changes in lung structure) will also facilitate cross-species extrapolation.

HERL researchers in pulmonary toxicology will continue to emphasize the development of chemical-specific information in support of the national ambient air standards (e.g., ozone, NO_2, and acid aerosols) as well as regulations for hazardous air pollutants (e.g., methanol, volatile organics). These research areas represent the progression of the program toward the development of predictive models for dose-response assessment. For laboratory animal studies, greater emphasis will be placed on relating tissue dose to early indicators of chronic lung disease. The human clinical studies program will experience some growth in the areas of lung biology and dosimetry. Future research directions in pulmonary toxicology can be highlighted as follows:

- *Hazard Identification*

  - Develop *in vitro* methods for evaluating pulmonary toxicity using human cells

- Develop and refine methods to characterize lung dosimetry in humans including, deposition, absorption, metabolism, and elimination

- *Dose-Response Assessment*

  - Develop animal models for human lung disease (e.g., emphysema, aging, asthma)

  - Evaluate mechanisms of lung injury related to exposure parameters (intensity and duration), chronic lung disease, and role of inflammatory processes in both laboratory animals and humans

  - Evaluate concentration/time interactions

- *Chemical-Specific Data*

  - National Ambient Air Quality Standard pollutants (e.g., ozone, NO_2, acid aerosols)

  - High-production-volume chemicals (e.g., methanol, VOCs)

- *Cross-Cutting Issues*

  - Gather data on complex mixtures (e.g., alternative fuels, indoor air)

  - Develop biomarkers of lung injury

2.1.3.4 Immunotoxicology

Immunotoxicology is an emerging program in HERL developed under the Environmental Toxicology Division and the Human Studies Division, and it is designed to investigate the effects of environmental chemicals on immune function and to relate these effects to increased risk of infectious, neoplastic, allergic, and autoimmune disease. In its formative stages, this program focuses on developing and validating both *in vivo* and *in vitro* methods for hazard identification. This research will identify the most sensitive and predictive methods for screening and characterizing immunotoxicants and facilitate the development of Agency test guidelines. Especially important will be an understanding of the relationship of these test methods to actual compromised immune function (i.e., increased susceptibility to disease) and the significance to risk assessment.

This program will address the use of immunotoxicity test results in risk assessment. A few tests will directly measure (*in vivo*) host resistance, hypersensitivity, or autoimmunity in animal models. The issues involved in extrapolating the results to humans are the same as for any other type of toxicity data (e.g., extrapolation from animal to human or from high to low dose). For certain environmental toxicants, analysis of the relevance of many rodent exposure studies to human health effects awaits further investigation. HERL's ability to conduct immunotoxicological testing in both human (*in vivo* and *in vitro*) and animal models is unique. Well-designed studies conducted in parallel in humans and animals are being developed and applied. Epidemiological studies and research designed to assess chemical immunomodulation in man are necessary to confirm the animal data. This combined approach will result in a better understanding of immunotoxic effects of pollutants, and facilitate the risk assessment process based on immunotoxicological data.

Research to validate test methods will include studies to understand mechanisms of action for immunotoxicants, local versus systemic effects as they relate to route of toxicant exposure, species dependence, and genetic influence on susceptibility. Biomarkers will also be developed that are predictive of enhanced susceptibility, severity, and/or recovery from disease. The development of host resistance models will lead to a better understanding of how exposure to toxic chemicals affects susceptibility to disease as a result of suppression or unwanted stimulation of the immune system.

Future research directions in immunotoxicology can be highlighted as follows:

- *Hazard Identification*

  - Develop models of infectious, neoplastic, and allergic disease

  - Develop and validate methods for screening and characterizing immunotoxicants both *in vivo* and *in vitro*

- *Dose-Response Assessment*

  - Examine mechanisms of immunotoxicity

  - Develop models for evaluation of sensitive populations

- *Cross-Cutting Issues*
  - Develop biomarkers that reflect immunocompetency

2.1.3.5 Pharmacokinetics

The pharmacokinetics program within the Environmental Toxicology Division is designed to explore the quantitative relationships between exposure and integrated dose to the target tissue. Research efforts include the quantitative study of absorption, distribution, metabolism, and elimination of pollutants and the use of mathematical models to describe these processes. This program area, which is new for HERL, will experience significant growth in the coming years. The two major areas of research will be characterizing dose to the target site for various environmental chemicals (hazard identification) and developing predictive models of dose (physiologically based pharmacokinetic models) for dose-response assessment.

Researchers will focus on developing methods and collecting data to characterize dose to the target site for a variety of toxicant classes of special interest. These data are needed to develop predictive dose models and to improve the accuracy of risk assessment. Research efforts will also develop and improve biomarkers for use in characterizing dose to the target tissue. Efforts to collect more experimental and physiological data relative to effective dose will be coupled with studies on toxic mechanisms of action to reduce uncertainties inherent in the use of external exposure information for risk assessments.

Experimental dosimetry studies will also begin to examine the influence of route, duration, chemical matrix, and rate of exposure on delivered dose to better understand the uncertainties inherent in extrapolating data from one exposure scenario and species to another. These data will be used to evaluate theoretical dosimetry models to predict target dose of inhaled gases and ingested or absorbed chemicals in humans and laboratory animals. Consideration will be given to defining factors (both physiological, physicochemical, metabolic, and anatomical) that influence pharmacokinetic parameters including structure-activity relationships.

Mathematical formulations for the deposition of compounds following oral, dermal, or inhalation exposure will be evaluated using data collected from laboratory animals and humans on absorption, metabolism, distribution, and elimination of parent compounds and their metabolites. These models will provide for better predictions of dose-equivalency across species and across exposure conditions.

Until the recent reorganization of HERL, small pockets of pharmacokinetic research were ongoing but were not well coordinated. The reorganization has resulted in an integrated program in pharmacokinetics that will experience major growth in the coming years. Expansion of the program will focus on developing the scientific expertise to measure toxicants in biological tissue, evaluating pharmacokinetic properties including their relationship to health outcomes, and developing the predictive models needed for dose-response assessment.

Future research directions in pharmacokinetics can be highlighted as follows:

- *Hazard Identification*
  - Develop data on pulmonary absorption in the lung, including improved morphometric data
  - Develop and validate *in vivo* and *in vitro* methods for studying dermal and oral absorption
  - Develop and validate *in vitro* and *in vivo* methods for studying elimination (metabolism and excretion) of toxicants
  - Expand efforts to characterize dose to target tissue
- *Dose-Response Assessment*
  - Place increased emphasis on developing extrapolation models for dose, particularly cross-species, route-to-route, and high-to-low-dose and acute-to-chronic-exposure
  - Evaluate factors (e.g., age, sex, disease status) that influence target dose to determine the appropriateness of physiological, anatomical, and physicochemical parameters used in predictive models
  - Evaluate influence of elimination processes, including metabolism and excretion, on target dose
- *Cross-Cutting Issues*
  - Develop biomarkers of dose

2.1.3.6 Developmental and Reproductive Toxicology

The developmental and reproductive toxicology program is designed to explore the effects of environmental pollutants on both development (i.e., interference with developmental processes) and reproduction (i.e., the functional integrity of both the male and female reproductive systems).

In general, *in vivo* methods are available for detecting developmental toxicity, but little is known regarding the mechanism of this type of toxicity. Researchers will continue to develop and validate embryo organ cultures as *in vitro* tools for examining biochemical and molecular events associated with abnormal development. Research in the developmental area will become increasingly focused on interpretation and extrapolation of methods to characterize developmental effects. Reproductive studies, on the other hand, will focus more on methods validation.

An improved ability to interpret and extrapolate both developmental and reproductive effects necessarily relies on a better understanding of mechanisms. Efforts will be directed toward understanding alterations in endocrine control of reproductive function, reproductive dysfunction (including early pregnancy loss), and mechanisms of teratogenesis as well as toward examining the relevance of maternal toxicity on fetal well-being. Predictive models will also be improved by studies that link dose to the fetus to developmental outcome. Also, research to understand the pathological events in homologous developmental models will improve interspecies extrapolation of teratogenic effects.

These research areas reflect, to a large extent, the natural progression of research in developmental and reproductive toxicology from hazard identification to dose-response assessment. Growth in this program will occur primarily in the areas of mechanisms of teratogenesis (structure/function), ovarian physiology, and mathematical modeling of dose-response data. Less emphasis will be placed on the development of *in vivo* bioassays. Future directions in the program can be highlighted as follows:

- *Hazard Identification*
  - Develop *in vitro* models for evaluating mechanisms of developmental toxicants
  - Systemically evaluate structure-activity relationships for developmental toxicants
  - Evaluate the relationships between gamete production/function and pregnancy outcome
  - Complete development of alternative reproductive test
  - Develop cell cultures to better identify direct-acting reproductive toxicants
- *Dose-Response Assessment*
  - Evaluate physiological, cellular, and molecular mechanisms of developmental and reproductive dysfunction, *in vitro* and *in vivo*
  - Evaluate quantitative dose models for developmental and reproductive toxicity
  - Link administered dose to delivered dose to outcome for both developmental and reproductive endpoints
  - Evaluate the influence of critical periods and maternal toxicity on developmental outcome
  - Identify and evaluate the models for susceptible populations (including the aged)
  - Elucidate the role of endocrine factors in reproductive toxicity

2.1.3.7 Epidemiology (Human Studies)

The epidemiology program is designed to explore the magnitude of health risks associated with exposure to environmental pollution through studies of humans in their natural environment and to refine methods for conducting this research. The products of epidemiologic studies provide data that help to confirm hazards to human health, to illuminate health hazards not identified experimentally (e.g., by clinical, animal, or *in vitro* research), and to clarify the relative importance of hazards to the population. The HERL epidemiology research program takes advantage of collaborative interactions with investigators in the field who are associated with universities, public health departments, and other federal, state, or local agencies.

Epidemiology research has made important contributions to the regulatory and rule-making activities of the

Agency, especially relative to drinking water, water quality, air toxics, and pollutants for which the Agency has established National Ambient Air Quality Standards. A major thrust of the HERL program is to integrate information from diverse sources (e.g., clinical, *in vitro*, animal, and field work) to develop research programs that effectively address questions of environmental relevance. In addition, new projects have been initiated to develop and validate biomarkers of dose and effect that can be used to study the impact on individuals and populations of exposure to toxic materials in the environment. Future directions in environmental epidemiological research can be highlighted as follows:

- *Hazard Identification*
  - Develop and implement techniques to evaluate public health impacts of drinking water disinfection
  - Develop and validate methods to model and test study design as field studies are being developed
  - Develop estimates of human exposure and dose that can be used to establish correlations to effects observed in human populations in epidemiology studies
- *Dose-Response Assessment*
  - Identify populations that are particularly sensitive (responsive) to ozone in the environment
  - Determine the magnitude of risk associated with exposure to air-borne pollutants in individuals and populations
  - Determine the association between an acute response to pollutant exposure and the development of chronic conditions
- *Cross-Cutting Issues*
  - Develop and validate biomarkers of exposure and effect for use in human populations naturally exposed to toxic materials

2.2 NEUROTOXICOLOGY DIVISION

Most biological manifestations of mammalian life"including muscular movements, the capability to reproduce, respiration, cardiac function, sensation, perception, and cognition"are controlled by the nervous system, which is composed of the brain, spinal cord, and a complex network of nerves and supporting cells. The proper functioning of the nervous system is essential for health and productive life. One of the major functions of the nervous system is to transmit information or commands from one component of the body to another; this function is accomplished by a complex interaction of various kinds of nerve cells. Interruption of this process can result in a variety of subtle effects, such as lowering of IQ scores in children; indications of neurological dysfunction, such as paresthesias in the extremities, muscle weakness, or seizures; or neurodegenerative states resembling naturally occurring neurological diseases such as Parkinson's and Alzheimer's diseases.

Figure 2-4 is a simplified representation of the nervous system. Like other organs, the nervous system serves to maintain homeostasis under certain input/output conditions. Information concerning the external and internal environment is relayed to the central nervous system (CNS) by afferent neurons using chemical messengers (neurotransmitters). Processing of this information by the CNS may or may not result in output, which is transmitted by efferent neurons also using chemical messengers. Output from the CNS is usually seen as a changed motor function or neurohumoral status. A chemical may interfere with these input/output relationships, either by directly affecting the level of neuronal organization or by interacting with another organ and, thus, affecting nervous system function indirectly. The full range of neurotoxic changes reported in humans include motor, sensory, cognitive, and autonomic disturbances; and these effects are studied at the neurobehavioral, neurophysiological, neurochemical, and neuroanatomical levels of neural organization.

Neurotoxicants are chemicals, drugs, or other agents that interfere adversely with the function of the nervous system. There are many chemicals to assess for neurotoxicity: EPA receives approximately 1,500 notices of intent to produce new substances each year and 65,000 chemicals are already listed in the EPA inventory of chemicals. Estimates concerning how many of these chemicals are neurotoxicants vary by the class of chemical in question and the exposure scenario. For example, of the more than 1,400 active pesticide ingredients registered by EPA, more than half are considered to be neurotoxic.

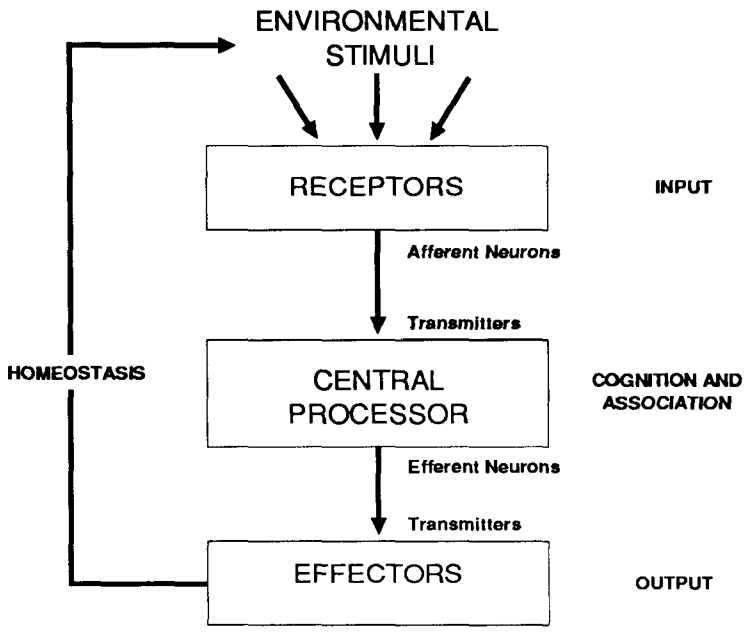

Fig. 2-4:
Simplified Representation of the Nervous System

Some estimates suggest that only 3-5 percent of industrial chemicals, excluding pesticides, have neurotoxic potential, although 28 percent of industrial chemicals for which there are occupational exposure standards have demonstrated neurotoxic activity. As a result, estimates of potential neurotoxicants in the EPA inventory range from 2,600 to over 18,000 chemicals.

Chemicals causing neurotoxicity include simple elements (e.g., lead, mercury), biological neurotoxins (e.g., botulinum toxin, tetrodotoxin), and synthetic compounds (e.g., DDT and industrial solvents). In humans, neurotoxic agents can cause a number of adverse effects on the nervous system, including impairment in muscular movement, alterations in sensation, deficits in learning and memory, mood and personality changes, and disruption of autonomic function.

Perhaps one of the earliest recognized neurotoxicants is lead, which is contained in industrial emissions, leaded gasoline, lead-based paints, foods, and beverages. Relatively low levels of lead have been shown to impair cognitive function in children. Another potent neurotoxicant is mercury, which was implicated in an environmental catastrophe during the 1950s in Minamata, Japan. Methylmercury formed from an industrial effluent became concentrated in fish and shellfish, which were eventually consumed by local inhabitants. Common signs of mercury exposure included a lack of coordination, speech impairment, and visual problems; functional deficits were eventually associated with specific neurohistopathological alterations. A different type of neurotoxicant is the mixture of chemicals known as the polychlorinated biphenyls (PCBs), which are stable, lipophilic industrial compounds known to cross the placenta and intoxicate the fetus. Some children of women exposed to PCBs during pregnancy have been developmentally impaired (e.g., hyperreflexia at birth, delays in psychomotor development, and deficits in visual accommodation).

Thousands of chemicals are produced for industrial use, and exposure to some of these chemicals can produce a chemically induced cetral-peripheral dying-back axonopathy, including muscle weakness, alterations in fine motor control, and paresthesias (e.g., abnormal prickling, tingling) in the extremities. Solvents are often used in glues, cements, and paints, and the possibility of industrial exposure is high. In addition, toluene-based spray paints, various solvents, and modeling cements are sometimes abused, resulting in intoxication. Cases have been documented in which excessive abuse of solvents has led to permanent neurodegeneration. Another class of chemicals for which the potential for exposure is high is pesticides, which include insecticides, rodenticides, and herbicides. Workers exposed to pesticides display obvious signs of poisoning, including tremors, weakness, ataxia, visual disturbances, and short-term memory loss. Exposure to some organophosphate pesticides can result in a delayed neurotoxicity, including irreversible loss of motor function and associated neuropathology.

Recently, concern has been expressed over the possibility that progressive neuro-degenerative diseases such as Parkinson's disease may be related to pesticide exposure, and that Alzheimer's disease may be related to aluminum concentrations in drinking water. Even more recent concerns have focused on possible neurobehavioral effects in children exposed to pesticides via the diet. Ample evidence links environmental chemicals with neurotoxicity in humans; thus, there is a need to identify and regulate such agents in the environment.

2.2.1 Divisional Program

The overall objective of the Neurotoxicology Division (NTD) is to provide the scientific basis and technological means for predicting, with a minimum of uncertainty, whether an environmental agent will produce neurotoxicity. The approach taken to this problem is to model human neurotoxic disease in laboratory animals and then to use the data collected in animals to make predictions about possible neurotoxic risks in humans.

2.2.2 Division-Specific Research Needs

2.2.2.1 Hazard Identification

Procedures to assess sensory, motor, autonomic, and cognitive function in animal models need to be developed, validated, and refined to provide the basis for extrapolation to human neurotoxicology. NTD also needs procedures that will lead to the quantification and categorization of developmental neurotoxicity. Little is known about the potential for chemicals to produce long-term, residual alterations in the function and/or structure of the nervous system following exposure in the early stages of development. In addition, NTD needs short-term *in vivo* or *in vitro* tests to identify cellular and molecular changes that are indicative of neurotoxicity. At the present time, initial screening procedures are less than ideal due to a lack of sensitivity to some neurotoxic effects (i.e., sensory deficits and cognitive dysfunction), the labor-intensive nature of the tests, and the relatively large number of animals required to achieve a statistically reliable answer. Procedures based on biochemical endpoints or using more recent advances in *in vitro* technology or molecular neurobiology need to be developed, validated, and refined.

2.2.2.2 Dose-Response Assessment

Dose-response data generated in animal models must be interpreted through acute and repeated dosing scenarios and measures of sensory, cognitive, and motor function. The use of homologous models to study chemical-induced neurotoxicity would greatly improve the risk assessment process. A better understanding is needed of inter- and intraspecies sensitivities, especially as they are related to differences in metabolism and/or distribution of neurotoxicants in the body. In addition, sensitivities of various age groups differ. Research in this area should also emphasize structure-activity relationships and mechanism of action to the extent that the information would be useful in the interpretation of dose-response assessments.

Another area in which research is needed is the analysis of the neural substrates that underlie some functional endpoints of neurotoxicity. A neurotoxicant-induced change in function implies that the chemical has interfered with the neural (i.e., neurochemical, neurophysiological) or anatomical substrate that mediates or controls the functional endpoint. The magnitude or nature of the functional change may be dependent on the portion of the neural or anatomical substrate mediating the functional change. A better understanding of these substrates will lead to a reduction in the uncertainty associated with risk assessments that use those endpoints.

Specific environmental factors, including temperature and stress, play a role in the manifestation of neurotoxicity. Little is known about the importance of temperature in this process; however, because many toxicants seem to interfere with thermoregulatory mechanisms, this endpoint might be a useful index of toxicant exposure. In terms of stress, NTD is particularly interested in the possibility that stress produced by exposure to a neurotoxicant or as a result of the neurotoxic effect can add to or synergize the effects of the neurotoxicant. In addition, the compensation or adaptation that sometimes occurs following repeated exposure to neurotoxicants is well documented. Studies that lead to a better understanding of compensation would be valuable in the risk assessment process because establishing thresholds is frequently difficult due to the compensation that occurs after the first few exposures. Compensation and adaptation are sometimes confounding variables in the setting of tolerance limits.

2.2.2.3 Chemical-Specific Data

NTD needs to quantify the dose-dependent effects of specific agents that may be of interest to the division and the program offices. Such chemical-specific studies could be useful for the continual validation and refinement of functional and structural endpoints. NTD has in the past provided data on specific agents, including xylene, styrene, the acrylates, substituted pyridines, and triphenylphosphite.

2.2.2.4 Biological Markers

NTD recognizes the need to assist in developing and validating biological markers of exposure and effects in human populations. These studies should provide biological markers that identify possible health hazards, document exposures and assess the efficacy of risk reduction

strategies, and provide a scientific basis for interpretation of biomarker data submitted to the Agency.

2.2.2.5 Pollutant Mixtures

Risks associated with human exposures to pollutant mixtures also need to be identified and assessed. Such research would determine the applicability of simplifying assumptions to risk assessments of chemical mixtures and compare the potency of various classes of pollutant mixtures using several bioassays. Of particular interest is the development of a strategy for hazard identification with these chemicals, i.e., one agent at a time, combinations of selected agents, or the composite material obtained at a specific site. The ability to assess the potential interactive nature (i.e., additive or synergistic) of specific agents in the mixtures is vital for reducing uncertainties in this area.

2.2.3 Research Plan

2.2.3.1 Hazard Identification

NTD has adapted a tiered approach to the study of chemicals for neurotoxicity. This stepwise progression from simpler to more specific and sophisticated assessments begins with a basic battery of tests to determine if further studies are necessary. In NTD, the first-tier *in vivo* functional observational battery (FOB) and motor activity tests have been developed and, as a battery, are currently being validated. The FOB consists primarily of a neurological examination for rodents, which is done in a systematic and sequential fashion. It measures chemical-related effects on sensory, motor, and autonomic function and can be used to study dose- and time-dependent effects in acute or repeated dosing protocols. The resulting profile of neurological effects is expected to have diagnostic value in the evaluation of unknowns. Research in this program will continue to validate systematically FOB and motor activity tests using known neurotoxicants that produce diverse neurologic syndromes.

NTD will also validate second-tier tests used to confirm an agent's effect on the nervous system and to provide a more precise estimate of neurotoxicity for assessing risk and setting exposure limits. Known neurotoxicants will be used to assess the usefulness of the tests, which are thought to measure specific structural and functional defects in the nervous system. From the resulting data, NTD will define which tests should be included in the second-tier analysis of neurotoxicity.

The mammalian nervous system is composed of heterogeneous cell types that serve as diverse and unpredictable targets of toxic insult. NTD research efforts will continue to develop, validate, and refine techniques for routine neurotoxicological assessments (e.g., those using novel neurotypic and gliotypic proteins as biochemical indicators of neurotoxicity) that are capable of detecting damage to diverse cell types. This research will be undertaken to develop and validate, using known neurotoxicants and negative controls, a number of neurotypic and gliotypic assays for detecting cellular and subcellular targets of neurotoxic attack. Identification of gene products associated with injury-induced changes in protein markers should also permit the diagnosis of neurotoxicity and ultimately lead to the development of *in vitro* assays. In addition, NTD will develop a stategy concerning the use of *in vitro* screening data within a larger overall screening effort.

NTD places a high priority on identifying and characterizing the potential effects of chemicals on the developing nervous system. Both functional and structural endpoints of neurotoxicity are of interest. The developing organism is preferentially vulnerable to some neurotoxicants, and the influence of developmental exposure on functional endpoints such as learning and memory must be assessed. Thus, some NTD research efforts are focused on developing and validating a model of cognitive development in an animal model. This research is designed to obtain tests of cognitive function in animal models that are homologous with tests used on humans infants. Then, researchers will validate the sensitivity of the animal model tests in identifying and characterizing cognitive dysfunction following the animals' developmental exposure to neurotoxicants.

Researchers will also focus on detecting and characterizing toxicant-induced anatomical damage to the developing nervous system. A number of developmental issues will be addressed, including the influence of maternal stress on postnatal function and the role of critical periods of exposure in the manifestation and persistence of developmental neurotoxicity. This program is based on the premise that functional deficits resulting from developmental toxic exposure are ultimately linked to alteration in neural connectivity, and that alterations in neural connectivity result from the disruption of certain developmental processes that are particularly vulnerable to toxic exposure. NTD will develop a test battery with known developmental neurotoxicants for a number of endpoints, including functional (sensory, motor, and cognitive function) and neuroanatomical measures. The long-term objective of this research is to establish the validity of a

number of anatomical markers that are indicative of exposure to neurotoxic agents in the developing organism. In addition, this work aims to establish the functional consequences of toxicant-induced alterations in connectivity. Eventually, this effort will lead to the development and validation of indicators of neurotoxic exposure in humans.

Although NTD can perform routine assessments of material for neurotoxicant-induced structural alterations, such endpoints are generally insensitive to early and subtle changes. Nor do they address neurological integrity over the range of sub-neuropathic doses or at earlier time points, when overt anatomical damage is not present and, if present, may be reversible. NTD research will focus on the use of gene probes/products of neural substances associated with events that are specific to regeneration and/or degeneration following toxic exposure. Such an approach should permit a sensitive, quantifiable measure of the progression of toxicant-induced nerve damage and could lead to the development of an *in vitro* screening procedure for neurotoxicity.

To understand the physicochemical properties that cause neurotoxicity, NTD will examine the structure-activity relationships of selected series of neurotoxic compounds. The procedures used will depend on the chemical class and type of neurotoxicity produced.

2.2.3.2 Dose-Response Assessment

A number of NTD research efforts focus on obtaining dose-response data in acute and repeated dosing paradigms. FOB/motor activity, reflex modification, visual evoked potentials, and schedule-controlled behavior procedures have been developed. These procedures have been validated to the extent that dose-response data could be used to evaluate the applicability of various risk assessment scenarios, including the standard no-effect-level approach, and effective dose values could be extrapolated from dose-response curves. NTD will place a high priority on the generation of complete dose-response curves for several different functional measures for a select population of prototypic neurotoxicants in order to compare the effectiveness of the various risk assessment scenarios. A number of procedures developed in NTD will also be used to evaluate structure-activity relationships routinely, including the FOB/motor activity first-tier screen, visual evoked potentials, reflex modification techniques, schedule-controlled behavior, and neurotypic/gliotypic protein markers. The information derived from such studies will lead to more accurate assessment of risk associated with exposure to chemicals.

Research in NTD uses sensory evoked potentials to measure stimulus-elicited changes in the electroencephalogram recorded from sensory areas of the brain. Because visual changes are the most frequently reported sensory effects, NTD research will focus initially on the use of visual evoked potentials to study dose-related neurotoxicity. The overall objective of this research effort is to use tests of visual function having known relationships to visual perception and to structural and functional segments of the visual system. Research in NTD will also focus on the brainstem evoked response (BAER) to study auditory dysfunction in rats. BAER is used widely in neurology, otology, and audiology to detect and diagnose auditory and, in some cases, more general central nervous system dysfunction. This research is intended to develop a validated test of auditory dysfunction in rats.

Another research emphasis is the use of behavioral procedures to study chemical-induced sensory dysfunction. The reflex modification procedure will be validated in laboratory rats for the auditory reflex using known ototoxicants, and the results will be compared to data gathered on other neurotoxicants having no ototoxicity. Once the procedure has been validated for auditory function, a multisensory procedure will be used to determine the usefulness of the technique in measuring neurotoxicant-induced changes in other sensory modalities.

The central nervous system is essential for directing attention to important environmental events and for acquisition (learning) and retention (memory) of such information. At present, no data suggest that either a functional observational battery or tests of motor activity used in a first-tier screen will provide information on whether a compound alters cognitive function. NTD research will compare simple, rapid tests of learning and memory to identify potential screening tests for learning and memory. The effectiveness of these procedures will be assessed by evaluating a number of positive and negative control compounds in terms of relative sensitivity and selectivity of effects. These studies will determine dose-related effects of the chemicals given under repeated, as well as acute, dosing regimens. The intended result of this work is a fully validated behavioral battery of tests for toxicant-induced cognitive dysfunction that can be used at the first tier of testing.

Methods are needed to characterize dose-related effects of neurotoxicants on cognitive function. Using known neurotoxicants, research in this area will develop and validate tests in rodents for the quantification of learning (automaintained reversal, repeated acquisition in the

radial maze), memory (spatial delayed response, nonspatial delayed response) and attention (signal detection). This effort is directed to reaching a better understanding of the neurobiology of cognitive behavior that will facilitate extrapolation of data from animal models to humans. Homologous models and/or fully validated models of cognitive function will provide the necessary basis for establishing safety factors in estimations of risk to human health.

The limbic system plays a role in mediating convulsions and seizure disorders, which are two common responses following acute exposure to higher levels of toxicants. Limbic structures show a predilection for the initiation and spread of epileptiform activity. It is possible that at lower levels of exposure to some chemicals, epileptiform activity occurs in the brain at doses below those required to elicit more severe behavioral manifestation of seizures. Thus, repeated low-level exposure to convulsants may lower seizure thresholds, sensitizing the brain and promoting the initiation and spread of seizures from the limbic system to other regions of the brain. Research in this area seeks to examine the selectivity of the brain site and nature of the neurotoxicant-induced disruption of limbic forebrain function that may underlie ensuing seizure disorders.

Numerous toxicants are known to produce motor dysfunction in experimental animals and humans. Study of chemical-induced motor dysfunction is carried out with a variety of techniques and in a number of testing scenarios, including the development of dose-effect and time-course characterization of toxicants, comparisons across toxicants, and the determination of the mechanisms by which toxicants affect motor function. Using positive and negative controls, researchers will validate the parametric conditions that are important for optimal testing and use appropriate testing procedures to determine the relationship between acute and repeated exposure effects, particularly in regard to compensation. Schedule-controlled responding will be used to study the mechanism of action of selected neurotoxicant agents, particularly those that produce a dying-back axonopathy.

One of the most common characteristics of neurotoxicants that affect motor function is that they can cause a degeneration of the axon. NTD researchers will study the effects of neurotoxicants on the transport of material in the axon by measuring the time-dependent distribution of radiolabelled materials injected into it. Project goals include the development of a relatively simple and noninvasive method to study the retrograde transport of materials, determination of the effects of various known neurotoxicants on retrograde transport, and the development of a similar procedure for the study of retrograde transport in the peripheral nerves of humans.

The metabolism of inositol phosphates (IPs) represents an important and vulnerable step in signal transduction in nerve cells. Initial studies have shown that IP turnover in the sciatic nerve of hens treated with organophosphate compounds is altered within days of exposure, and some of these changes are directly related to neuropathic effects rather than inhibition of acetylcholinesterase. Therefore, determination of IP metabolism may be a relatively simple biochemical marker for organophosphate-induced neuropathy that will be independent of cholinesterase inhibition. This work is intended to develop more sensitive and earlier tests for impending peripheral neuropathies that could augment the traditional, less sensitive tests now used in regulatory programs.

Several projects within NTD could ultimately result in the application of *in vitro* techniques, including the use of neurotypic and gliotypic protein markers and gene probe analysis, to the assessment of dose-response and structure-activity relationships. Additional *in vitro* techniques are needed for neurotoxicity testing, including some variant of currently existing techniques (i.e., primary cultures, cell lines, or cloned cells). Although few studies in NTD have been performed solely to study mechanism of action, work in this area could be key to decreasing uncertainty in the risk assessment process.

To perform such studies, the skill mix at NTD must be changed or augmented. One area of potential interest is the neurotoxicant-induced changes in ionic fluxes. Many neurotoxicants adversely affect neuronal function by interfering with membrane biophysics or the normal opening and closing of ionic gates located in the membrane. Voltage or patch clamp techniques would permit the study of many types of neurotoxicants, both in terms of their mechanism of action and structure-activity relationships. Another important research issue concerns the underlying neural substrate that mediates functional measures of neurotoxicity. Because information in this area would facilitate the interpretation of neurotoxicant-induced changes in these endpoints, work in this area is another means of reducing the uncertainty in risk assessment. At the present time, several functional endpoints have been developed and show sufficient promise, particularly in the area of learning and memory, evoked potentials, reflex modification, and brainstem evoked potentials.

NTD currently has no capability for determining the levels of parent compound or metabolites in regions of the brain or periphery. During the next three to five years, this capability will either be developed within NTD or through the on-site contractor mechanism. NTD currently conducts studies concerning responsiveness of different strains of rats to selected prototypic neurotoxicants in the FOB and motor activity evaluations. These studies will provide the basis for designing future studies. Future work in this area will also be concerned with the development of tests or a strategy for testing nonrodent species. NTD is also interested in the differential susceptibilities of different populations to neurotoxicants.

Stress and the changes associated with stress influence the manifestation of neurotoxicity by affecting the absorption, metabolism, and elimination of chemicals. Research efforts will examine the influence of nonspecific stress on dose-response curves to neurotoxicants; this work could provide useful information concerning high-to-low-dose extrapolation. Another important issue revolves around the possible adverse effect of adrenotoxic agents on brain structure and function. Research in this area will use known neurotoxicant and adrenotoxicants to damage components of the hypothalamic-pituitary-adrenal axis and determine the response of the system to toxic injury. This information is critical for understanding the interaction between stress and the manifestations of neurotoxicity"a necessary link for reducing uncertainty in the risk assessment process.

In terms of the influence of temperature on the manifestation of neurotoxicity, NTD research will compare the consequences of acute versus repeated exposure to prototypic neurotoxicant agents at various temperatures and investigate the mechanism by which selected neurotoxicants can influence the thermoregulatory system. This work is directed toward the gathering of data that permit the extrapolation of thermoregulatory effects of xenobiotics seen in laboratory animals to humans, thereby providing a basis for limit setting.

NTD will develop a strategy for studying the compensation and adaptation that accompanies repeated exposure to some neurotoxicants. Initially, studies will focus on the use of a functional endpoint having a stable baseline of responding, such as schedule-controlled behavior, and superimpose repeated dosing to known neurotoxicants on the baseline. The rate and extent of compensation that occurs will be measured; and strategies to reveal underlying homeostatic changes, such as the application of pharmacological drug challenges, will be explored. The overall objective of this program is a strategy for studying the role compensatory processes play in the expression of neurotoxicity; such information can be used to reduce uncertainty in the risk assessment process.

2.2.3.3 Chemical-Specific Data

NTD can study specific chemicals at several levels of neural organization. Routine questions concerning the capability of an agent to produce neurotoxicity can be studied using the FOB and motor activity tests; while more focused questions concerning sensory, motor, or cognitive deficits can be studied with existing procedures.

The search for new and more efficient fuel products has accelerated in recent years. Considerable concern has been raised about the increasing use of methanol as an energy source. NTD will conduct research on the potential neurotoxicity of methanol. If other alternative sources or additives are developed, further research will have to be conducted to support the hazard identification process and to characterize the chemicals for potential neurotoxicity.

2.2.3.4 Biological Markers

Using gliotypic and neurotypic protein markers as indices of neurotoxicity may eventually lead to the development of a biomarker for neurotoxicant exposure. Other approaches to the development of such a biomarker show promise and could be explored, perhaps through cooperative agreements. For example, some neurotoxicant agents (e.g., hexane or acrylamide) might produce an antigenic reaction after exposure. Future work could be directed to determining the nature of the antigenic reaction to these molecules and developing a simple, rapid test that could routinely be used in the work place or environment after exposure has occurred.

2.2.3.5 Pollutant Mixtures

Currently, NTD is not conducting research on chemical mixtures. There is a clear need to study the principal components of some chemical mixtures, particularly with respect to possible additivity or synergism of some of the components. Because many of the mixtures have large amounts of known neurotoxicants"for example, metals and organic solvents"this area of research would have a high priority. A critical need in this area is the development of a rapid, sensitive *in vitro* test, possibly based on the use of cell cultures, to rapidly screen mixtures and their components for potential neurotoxicity. The FOB

and motor activity tests should also be used to study chemical mixtures.

2.2.4 Emerging Issues

A number of major issues could change the priorities of the NTD program in the future. These include concerns regarding microorganisms, environmental agents and neurodegenerative disease, and alternative fuels.

2.2.4.1 Microorganisms

Research is needed to determine whether a causal relationship exists between environmental exposures to microorganisms (i.e., bacteria, viruses, fungi, genetically engineered and naturally occurring microorganisms) and human health effects. The central nervous system might be particularly vulnerable to microorganisms, as shown by the susceptibility of the brain to the AIDS virus. Research efforts may be necessary to develop appropriate test methods for hazard identification and to support a quantitative health risk assessment.

2.2.4.2 Environmental Agents and Neurodegenerative Disease

Both environmental agents and naturally occurring substances can affect the nervous system, resulting in neurodegenerative disorders. Possible diseases include amyotrophic lateral sclerosis, Parkinsonism, and an Alzheimer-like dementia syndrome. The last of these has been linked to the ingestion of products made from the false sago palm, which contains agents that resemble excitatory amino acids (which are neurotoxic in animal models). The discovery of this link between a naturally occurring compound and a neurodegenerative disease has stimulated the search for other toxic substances; and it is now known that other foods contain neurotoxic substances that may be related to the etiology of neurodegenerative disorders. Future research in this area may prove fruitful for identifying environmental links between exposure to chemicals and the development of progressive, neurodegenerative diseases such as Parkinson's and Alzheimer's diseases.

2.2.5 Summary

2.2.5.1 Hazard Identification

NTD will continue to systematically validate a first-tier *in vivo* screen for the assessment of chemical-related effects on sensorimotor function in rodents. *In vitro* procedures for routine neurotoxicological assessments will also be developed and validated. Focusing on the second tier of toxicological assessments, researchers will then define which currently available tests are appropriate for inclusion at this level.

In the area of developmental neurotoxicity, NTD will develop and validate methods to identify and characterize developmental toxicity. After developing and validating a model of cognitive development in an animal model, researchers will also validate the sensitivity of animal model tests in identifying and characterizing cognitive dysfunction.

NTD will also develop and validate cellular and molecular endpoints as indicators and predictors of neurotoxicity. This research will exploit the natural response of the central and peripheral nervous system to injury induced by chemicals. Research efforts will also be directed to using structure-activity relationships to understand the physicochemical properties of chemicals that cause neurotoxicity.

2.2.5.2 Dose-Response Assessment

Researchers will study mechanism(s) of action of neurotoxicants by examining the effects of agents at the cellular, subcellular, and molecular levels and then correlating those effects with neurotoxic signs measured in the whole animal. Of particular interest will be the interaction of metals or solvents with endpoints such as ionic fluxes inside and outside the cell.

Work will also emphasize issues associated with various extrapolations (i.e., route-to-route, high-to-low-dose, species-to-species). Special emphasis will be placed on experiments in which animals will inhale chemicals while they are being tested.

Because the body often compensates for or adapts to the effects of chemicals, NTD will develop a strategy for assessing the influence of these factors. Acute and repeated exposure to a prototypic class of neurotoxicants will be used to assess the underlying mechanism(s) of action. Also, NTD will examine the neurotoxicity associated with chemical mixtures. Known neurotoxic compounds will be tested in pairs, and the data compared to the results of testing individual compounds.

2.3 GENETIC TOXICOLOGY DIVISION

Interactions between genetic material and environmental agents can result in mutations. There are two types of mutations: 1) those that occur in germ cells (germ cell mutations), which may be passed on to the next generation, and 2) those that occur in somatic cells (somatic mutations), which are not passed on to the next generation. Genetic diseases, such as hemophilia, cystic fibrosis, and Tay-Sachs disease are examples of health effects associated with germ cell mutation; an example of a health effect due to a somatic mutation is cancer.

Germ cell mutations are rare and many, if not most, result in "carrier" individuals rather than diseased offspring; thus, no cases have been documented in humans of new genetic diseases induced by environmental exposures. Studies in rodents and other animals, however, clearly indicate that germ cell mutations can be induced by chemical exposure. It is thus theoretically likely that chemicals pose a threat both to individual offspring and to the integrity of the human genome and thus to future generations. While definitive proof is lacking, little doubt exists that many chemicals have the potential to induce transmissible human mutations.

By comparison, a large body of data suggests that environmental exposures can result in somatic mutations leading to the development of cancer. Various genes that control cell growth, proliferation, and differentiation have been implicated in the genesis of cancer. Activated cellular oncogenes have been detected in tumors from humans and animals, suggesting that cellular oncogenes are critical targets of carcinogens. In addition, the inactivation of certain tumor suppressor genes (anti-oncogenes) is postulated to result in the induction of cancer. Evidence also has accumulated implicating a class of agents that can cause cancer, yet do not appear to produce chemical damage to DNA. These agents have been called "nongenotoxic carcinogens."

In 1987, the International Agency for Research on Cancer (IARC) conducted an evaluation of the causal associations between exposure to carcinogens and human cancer. Since 1969, IARC has reviewed, assessed, and classified those agents, processes, occupational exposures, and industries to which humans are exposed. To date, IARC has classified 50 sources as carcinogenic to humans, including:

- Single agents (e.g., aromatic amines, cancer chemotherapeutic drugs)
- Mixtures (e.g., analgesics, coal tars/pitches, soots)
- Processes (e.g., aluminum production, coal gasification, iron and steel founding, coke production)
- Occupational exposures (e.g., petroleum refining)
- Industries (e.g., the rubber industry)

IARC has also classified another 37 agents as "probably carcinogenic to humans" and a further group of 150 agents as "possibly carcinogenic to humans."

Taken in totality, cancer and other diseases of genetic origin vitally affect human welfare. These diseases often result in debilitation, death, and human suffering. By some estimates, at least 10 percent of all human disease exhibits a significant genetic component. Exposures to new mutagenic agents in the environment could be increasing the number of individuals who are "carriers" of altered genes. Such an increase would lead to an increased incidence of cancer and genetic diseases in future generations.

2.3.1 Divisional Program

Research conducted by the Genetic Toxicology Division (GTD) addresses both mutation and cancer. The program is designed to explore the influences of environmental agents on 1) the processes of genetic change in somatic and germinal tissues (mutagenesis) and 2) the conversion of normal cells to neoplastic cells (carcinogenesis). All direct and indirect interactions between chemicals and the genetic material of the test organisms are of interest if they may lead to adverse health effects. Research efforts are also focused on the genetic toxicology of environmental chemicals and complex mixtures, including the toxicology of genetically engineered microorganisms and their metabolites.

The hypothesis that carcinogenesis results from genetic changes"the somatic mutation theory of cancer"is the basis for the study of genetic toxicology. Cancer is thought to progress from carcinogen exposure to tumor formation through a multistage process, including initiation, promotion, conversion, progression, and metastasis (see Figure 2-5). Germ cell mutagenesis, on the other hand, is highly cell-stage specific. A mutation in the germ line may occur in premeiotic or postmeiotic cell stages (i.e., before or after reduction division) and may result in mutated offspring or reduced fertility. Chemicals that

have the ability to induce germ cell mutations deserve special attention from a regulatory and a research perspective.

The somatic mutation theory of cancer also provides the rationale for the use of short-term tests to detect potential carcinogens. These tests may also be used to detect active metabolites of mutagens or carcinogens in body fluids and tissues, to identify genetically active components in complex mixtures, and to investigate possible mechanisms of carcinogenesis and germ cell mutagenesis. From a mechanistic perspective, both cancer and heritable mutation are complex. Studies in whole animals are essential to define the intricacies of chemical carcinogenesis and mutagenesis"to determine appropriate biological markers for cancer and genetic disease, to model the shape of dose-response curves for genotoxic as well as nongenotoxic carcinogens, and ultimately to refine estimates of human health risks.

identification, dose-response assessment, chemical-specific data, biological markers, and pollutant mixtures.

2.3.2.1 Hazard Identification

Short-term tests are efficient means of detecting DNA-reactive genotoxic chemicals. These tests, however, vary in the types of genetic damage that can be evaluated and in the specific classes of mutagens/ carcinogens that can be detected. Research is needed to define the capabilities and limitations of test systems. Short-term test databases and structure-activity relationship (SAR) methods are needed for specific chemical classes of concern to the program offices to 1) solve data needs and 2) provide the theoretical and practical bases for correlating molecular struc- ture with biological activity. In conjunction with these needs, research is required to identify metabolites in critical toxicologic processes. Depending on the specific nature of the data gap or SAR problem, GTD or other HERL divisions may perform research to generate the required data.

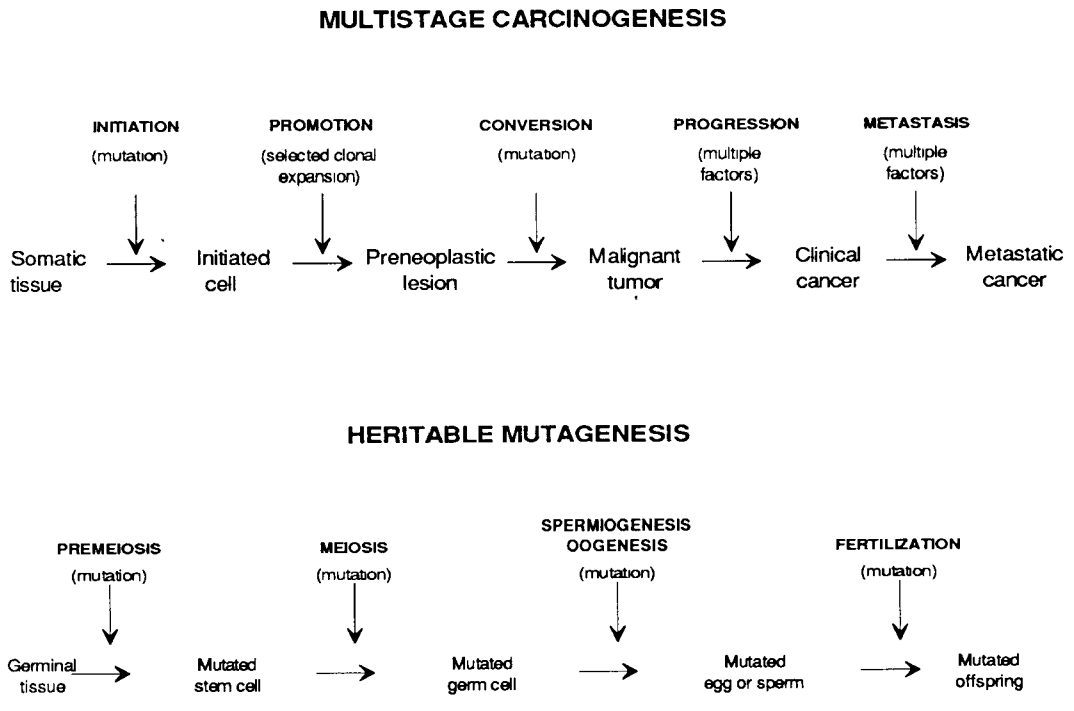

Fig. 2-5: Multistage Carcinogenesis

A major emphasis must be placed on the definitions of test system capabilities and limitations. Because certain substances seem to induce tumors without appearing to induce genotoxic effects, short-term tests are needed for nongenotoxic carcinogens—and specifically, for chemicals that are not genetically active in short-term mutagenesis screening assays but cause cancer at selected target sites in rodent bioassays. Additional effort should be focused on the development of tests that are relevant to rodent hepatic and renal carcinogenesis (and research is required to ascertain the human relevance of these cancers).

2.3.2 Division-Specific Research Needs

The most important questions GTD faces in the next three to five years are described in this section. These research needs are covered under the research topics hazard

Short-term *in vivo* and *in vitro* tests for tumor promoters need to be evaluated. One GTD project in this area deals with cell-cell communication as a short-term bioassay for nongenotoxic carcinogens. In addition, short-term *in vivo* tests are needed to detect tumor initiators as well as promoters. Such methods, based on an assessment of DNA damage (e.g., gastrointestinal tract nuclear anomalies or DNA strand breaks), can provide a higher level of confidence than *in vitro* approaches because they can account for factors such as species-specific tissue/organ sensitivity, pharmacokinetics, and pharmacodynamics.

The EPA Mutagenesis Guidelines address environmentally induced, potentially inheritable genetic damage and stress the need for improved means of identifying chemicals that may be hazardous to germ-line cells. The "standard" germ cell assays now available are relatively insensitive as chemical screens, and relatively few such studies of chemical effects on germ cells have been reported. Extrapolation of test results from somatic cell assays is limited by the processes/targets that are unique to germ cells. Hence, new short-term methods are needed to test for chemical induction of heritable genetic effects.

Other efforts should determine if genetically engineered microorganisms (GEMs) can survive in the gut, invade other tissues, and induce adverse health effects. Projects would be designed to identify genotoxic and metabolic changes that may occur as a result of direct exposure to GEMs or as a result of their use for bioremediation of hazardous spills and waste sites and in other environmental and sanitary operations.

2.3.2.2 Dose-Response Assessment

In both mutagenicity and carcinogenicity testing, the development of dose-response data and mechanistic information is critical for the quantitative evaluation of the biological effects considered in risk assessment models. Research efforts are needed to explore the extent to which dose-response investigations in both cell culture and rodent systems are applicable to humans at realistic environmental exposure levels, i.e., appropriate extrapolation models must be developed in support of the risk assessment process.

Mechanistic research efforts using molecular techniques should be designed to identify specific types of induced genetic damage. Mutation induction is a multistep process of initial lesion occurrence (perhaps from the formation of a DNA-chemical adduct), the repair or misrepair of that lesion, and the generation of daughter cells containing the mutation. Because different cell types and different animal species may repair DNA with different degrees of fidelity, mechanistic research also must include interspecies comparisons. Cancer induction involves many potential mechanisms (e.g., gene or chromosomal mutation, heritable changes in DNA transcription reflected in altered gene expression) and multiple stages. A variety of factors or conditions"including enzyme and hormone levels, rates of cellular proliferation, target cell specificity, and genetic predisposition"influence the expression of neoplasia. Therefore, to properly model the relationship between exposure to cancer-causing environmental agents and expression of the disease, researchers must explore the possible mechanisms of carcinogenesis and the factors that modulate neoplastic change.

One type of work that is needed is an examination of the relationship between critical biochemical processes and/or preneoplastic lesions and the development of cancer in experimental animals. These experiments have a twofold goal: 1) to develop data on mechanistic aspects of environmental carcinogens (both genotoxic and nongenotoxic) so that the risk associated with exposure to these chemicals can be assessed both qualitatively and quantitatively; and 2) to produce data for use in developing biologically based models for carcinogenesis (for quantitatively estimating carcinogen potency), and in qualitatively determining the appropriateness of particular oncogenic endpoints (e.g., mouse liver or rat kidney tumors) for human risk assessment.

2.3.2.3 Chemical-Specific Data

What chemical-specific information program offices need depends on their legislative mandates; however, considerable overlap exists among these requirements. GTD's capabilities for addressing chemical-specific information needs are generally applicable to all program offices. Specific chemicals of interest to various offices are as follows:

Drinking water: Products of disinfection and specific compounds for regulation

Air: Representative or index chemicals for broad chemical classes

Hazardous waste: Chemicals not represented in the Integrated Risk Information System (IRIS) or for which important data gaps exist

Superfund: Chemicals included in the Superfund comprehensive risk assessment for sites; data needs include reference doses, potency factors, and SAR information

Toxics: Chemicals that represent broad classes or conceptual assessment criteria

2.3.2.4 Biological Markers

A major source of uncertainty in risk assessment lies in estimating the extent of exposure. For genotoxic effects, the dose delivered to the cell DNA is critical. New methods are needed for measuring the internal exposure of experimental animals and humans to environmental genotoxic chemicals using biochemical entities (e.g., DNA and protein adducts and urinary metabolites) via ultrasensitive methods (e.g., GC-MS and ^{32}P-postlabeling).

An experimental basis must also be established for relating dose to the DNA (DNA adducts) to the induced genetic effects (sister chromatid exchange, chromosome aberrations, micronuclei formation, and gene mutation) that can be monitored in humans. Further research is required to establish the shape of dose-response curves and to develop biologically based dose-response models, especially for nongenotoxic carcinogens.

2.3.2.5 Pollutant Mixtures

Methods and approaches are needed to detect genotoxic agents as complex mixtures in ambient air and indoor air combustion sources, waste incinerators, environmental tobacco smoke, drinking water, wastewater, sludges, and products resulting from bioremediation. New methods are required to detect potentially carcinogenic chlorinated hydrocarbons, highly reactive gaseous species (e.g., PAN), and nongenotoxic carcinogens (e.g., diethylhexylphthalate). Additional effort should be directed to developing and applying green plant genetic test systems for on-site monitoring of chemical dump sites and other areas impacted by pollutant sources. After these tests are developed, their utility must be defined through *in situ* assessment and environmental monitoring.

Another area for research is modeling and testing the interactions of genotoxicants with other pollutants using artificial mixtures and actual fractions of environmental mixtures. Environmental substances that should be examined include drinking water, ambient outdoor and indoor air, industrial effluents, hazardous waste, and source emissions.

Major areas for GTD research effort have been defined as the development of methods/approaches for 1) determining which emission sources are the major contributors of carcinogens to ambient air (both indoor and outdoor); 2) estimating human exposure to complex mixtures, including biomonitoring source, ambient, microenvironmental, and personal samples, as well as *in situ* monitoring; and 3) determining the carcinogenic potency of complex mixtures.

These objectives, taken together, include continued research to test and further develop the comparative potency method for cancer risk assessment of combustion emissions.

2.3.3 Research Plan

2.3.3.1 Hazard Identification

Several new directions will be pursued relative to SAR and database development. Specialized databases for use in SAR chemical analysis will be assembled in the areas of genetic and developmental toxicology (with HERL's Developmental Toxicology Division). In collaboration with the Office of Pesticide Programs, data submitted by registrants will be computerized to provide similar database and SAR analytical capabilities.. In the area of computational SAR:

- Molecular similarity will be quantitated as it relates to molecular recognition and biological activity.
- Causal molecular models will be developed from computationally available molecular properties that predict the distribution, transformation, deposition, or biological activity of classes of chemicals.
- Mechanisms of action of nongenotoxic carcinogens will be explored by assessing molecular similarity indices.

Conventional short-term tests and test batteries for identifying potential genotoxic agents will be evaluated and adapted as necessary to efficiently detect specific classes of chemicals. To detect chemicals that induce aneuploidy in mammalian cells, new test methods will be developed. As new molecular techniques become available, this technology will be applied to develop assays that are capable of evaluating specific types of genotoxic damage. GTD investigators will develop short-term test methods to improve the detection of nongenotoxic car-

cinogens. Endpoints of immediate interest include alterations in gap junction proteins, alterations in gene expression, and morphological cell transformation.

The heritable mutation research program will be refocused in the next two years to utilize new recombinant DNA techniques for evaluating mechanisms of mutation in germ cells. The significance of genetic recombination and segregation events at meiosis leading to mutation will be analyzed in yeast, with the objective of extending these studies to the mouse. The long-range goal in this program is to achieve a better understanding of germline-specific targets and mechanisms in the induction of heritable mutations, and to improve analytical capabilities to detect such events.

Research on GEMs will be designed to evaluate various mutant and recombinant microorganisms and to identify any adverse effects they could have on human health. Efforts will be made to develop new and improve existing methods for monitoring exposure to engineered bacterial strains and/or genotoxic metabolites generated by these organisms. New research will detect alterations in the activity of biomarker enzymes and the effect of these changes on the biotransformation of xenobiotics. GTD researchers will give particular attention to changes leading to increased levels of toxic, mutagenic, or carcinogenic metabolites particular attention.

2.3.3.2 Dose-Response Assessment

The major issues associated with improving cancer risk assessment are:

- Improving the accuracy of the exposure assessment
- Comparing the mechanisms of action of chemicals in both rodent and human tissues
- Improving methods for extrapolating rodent cancer data to humans

Problems in these areas can contribute significantly to the potential uncertainty, unavailability of data, and inaccuracy found in current cancer risk assessment procedures. Designing and evaluating biological marker/molecular biological techniques for biological monitoring would greatly increase the accuracy of exposure assessment. In addition, understanding the mechanisms of action of chemicals and the differences in the mechanisms between rodents and humans will improve the basis for human risk estimation. Incorporation of the biology of cancer induction, promotion, progression, and metastasis into the risk estimation/extrapolation models will also improve the cancer risk estimates.

The use of internal or target dose measures such as DNA adducts or cytogenetic effects will improve the accuracy of dose determinations for interspecies extrapolation and will be useful in species-species and route-route extrapolations. The development of exposure-, dose-, and genetic effects relationships for genetically active chemicals will allow a more precise extrapolation of risk from rodent data to the human population. This will be evaluated with nonsite-specific carcinogens through systemic exposure. GTD researchers will determine the dosimetric relationships between exposure and dose to the target cells and induced genetic effects. As an example, peripheral blood lymphocytes will serve as target cells in rodents, and humans exposed to the cancer chemotherapeutic drug, diazaquone (AZQ), will serve as target tissues for this drug.

Uncertainty in carcinogenesis risk assessment arises, in part, from a lack of understanding about the underlying chemical and biological mechanisms that are responsible for the development of the cancer cell. Knowledge of mechanisms of action can assist in the choice of the most appropriate dose-response models for the extrapolation process. Mechanisms of action of carcinogens can be genotoxic or nongenotoxic in nature; therefore, the similarities and differences in the mechanisms of action of carcinogens in both rodent and human tissues need to be defined.

The induction of protein kinase c or other enzymes associated with nongenotoxic carcinogenesis, alterations in the expression oncogenes or tumor marker genes, and alterations in the structure of oncogenes will be measured by state-of-the-art molecular biological techniques. Alterations in gap junction intercellular communication has become recognized as an important element in responses to nongenotoxic carcinogen treatment in cells as they progress to a more tumorigenic state. GTD investigators will study the functional aspects of gap junction membrane components in fibroblastic and epithelial cells at the molecular, organelle, and cellular level using long-term cell culture systems. In addition, they will assess the involvement of oncogenes and oncogene products in early promotional events and intercellular communication. This work will lead to a better knowledge of mechanisms of action of different classes of nongenotoxic chemicals and will provide a more explicit basis for extrapolation model development.

A major advance in cancer research during the last 30 years has been catalyzed by the simultaneous recognition that:

- Carcinogenesis is a complex, multistaged biological process.
- Chemicals can increase the incidence of experimental cancer via a variety of separate, unrelated biochemical mechanisms, some of which are irreversible (e.g., tumor initiation) and others of which may be reversible. In theory at least, these latter mechanisms have thresholds for activity so that exposures below the threshold could result in no, or greatly reduced, harmful consequences.

Along with these advances in understanding has come the realization that the statistically based cancer risk assessment models (e.g., the linearized multistage model) now in use are probably not appropriate for all types of chemical carcinogens. This limitation has, in turn, prompted the development of biologically based dose-response (BB-DR) models for cancer risk assessment. BB-DR models are based on the quantification of certain preselected biochemical processes that are thought to be critical to the progression of carcinogenesis.

The recently developed Moolgavkar-Knudson-Venzon (MKV) model is one of the more widely used BB-DRs. It quantifies the carcinogenesis process in terms of a series of biological parameters, such as mutation rates in target cells and the rate of growth of various putative premalignant cellular lesions. This model has proved useful in explaining the incidence of a number of spontaneous human cancers. More research is needed to evaluate the feasibility of using BB-DR models to explain the incidence of chemically induced cancer in experimental animal models. The successful development and application of these models should 1) greatly facilitate extrapolations from experimental cancer data to human risk, and 2) permit the use of numerous types of biochemical data (e.g., measurement of DNA synthesis and preneoplastic lesions) in the cancer risk assessment process.

Several experimental approaches have been developed to determine interspecies sensitivity; all are based on comparing responses of humans, animals, or their tissues to carcinogen exposure. Humans and rodents are exposed *in vivo, in vitro*, or by a combination of the two (parallelogram approach); and the data are then compared. Interspecies sensitivity for selected classes of carcinogens can be determined by using the same agent and similar exposure conditions for both rodents and humans. When available, results from quantitative epidemiology will be incorporated to define the potency of a human carcinogen. An effort will also be made to define the potency of a carcinogen in an experimental situation using the same route of exposure as associated with the human data. Although the measurement of tumor formation is the most desirable endpoint, other endpoints that may be related to tumor formation will be used (e.g., genetic or cytogenetic damage).

When human exposure and effects information are not available or cannot be generated, GTD researchers will use human and animal cells or their tissues in culture (*in vitro*) to obtain interspecies sensitivity constants. Although the measurement of tumor formation is not possible *in vitro*, other endpoints related to the cancer process"DNA damage, gene mutation, chromosomal effects, and morphological cell transformation"can be used. The researchers will apply new molecular methods to understand the fundamental interspecies differences between the responses of rodent and human cells to genotoxic agents. A systematic evaluation of genotoxic response (including specific gene loci and differences in DNA repair) will allow a determination of how genotoxic effects in rodents extrapolate to similar effects in humans. GTD investigators will use molecular techniques (insertion of human DNA repair genes and specific target genes) to define and quantify the specific interspecies differences"a process that will reduce the uncertainty in the interpretation of *in vitro* and *in vivo* rodent data and allow a more realistic and accurate assessment of human risk from particular environmental exposures.

The parallelogram method examines responses from both *in vitro* and *in vivo* situations and combines four constants into a prediction of carcinogenic effects in man. Each one of these interspecies sensitivity/extrapolation constants requires a determination of the potency of the chemical under the treatment conditions. "Chemical potency" is a complex issue to resolve. GTD researchers will explore the development of a potency measure using a combination of dose, tumor multiplicity, tumor incidence, species, sex, site specificity, malignancy, and metastasis.

Lung cancer, which is associated with exposure to carcinogens in the lung, is a major cause of death. The primary target site for these carcinogens is the bronchial epithelial tissues, which are transformed by a series of dysplastic, metaplastic, and neoplastic changes. Methods for obtaining and culturing rodent and human respiratory tissues are needed to define interspecies sensitivity constants to respiratory carcinogens. After these procedures

are developed, they can be used to subject tissues to carcinogen exposures; and then various parameters of the expression of the neoplastic phenotype or of genotoxic injury will be measured in a comparative fashion. With these endpoints, interspecies extrapolation constants will be determined. Investigators will also use this research to assess the correspondence between mechanisms of neoplasia in humans and rodents, and to quantitate their similarities.

A major confounding factor in defining interspecies sensitivity to carcinogens is the large interindividual variation observed in the human population. In contrast, relatively small interindividual variation is observed in experimental animals. Human interindividual variation in response to carcinogens is crucial for defining interspecies extrapolation in the risk assessment process: The issue is whether to regulate a carcinogen based on the most susceptible human population or on the "average" human response. Interindividual variation will be examined using explanted tissues from surgical specimens from a variety of tissues.

Recent advances in molecular biology indicate that genetic changes are involved in the etiology of cancer. There should be, therefore, a set of biomarkers that signal the relevant genetic alterations, and these markers should be detectable before the emergence of cancer. Delineating such a set of mechanistically consistent biomarkers could significantly diminish the time needed to perform assessments of human carcinogenic activity. Furthermore, these assessments could be performed in the exposed human target tissues. If used to complement existing animal bioassays or lengthy *in vitro* transformation bioassays, biomarkers could significantly shorten the time and cost of such bioassays.

2.3.3.3 Chemical-Specific Data

GTD has the capacity to fully evaluate the mutagenic and oncogenic potential of agents of environmental concern, including pure chemicals and complex environmental mixtures. The process of evaluating potential environmental genotoxicants is frequently approached through the step-wise application of bioassay methodologies involving short-term screening tests, confirmatory short-term bioassays, and, eventually, established whole-animal mutagenesis and carcinogenesis bioassays.

Working collaboratively, scientists from HERL and the Office of Toxic Substances (OTS) have identified several chemical classes for SAR research. Chemical classes were selected according to the perceived need for understanding the chemical properties and structures responsible for the observed biological activity. The research team has evaluated three classes of chemicals (acrylates/methacrylates, anthraquinone dyes, and azo dyes) to pinpoint the structural properties responsible for their biological activity. These properties, which are applicable to other chemicals as well, will be used to evaluate the SAR approach to studying chemicals' biological activity.

GTD will also conduct research on the genotoxic potential of complex mixtures and on specific chemicals in drinking water, such as chlorinated organic pesticides; high-volume organic chlorinated hydrocarbon solvents; chlorinated phenols; chlorinated acids; disinfection byproducts of ozonation, chloramination, and a combination of the two; and complex mixtures of wastes and drinking water concentrates containing chlorinated organics. Such research is necessary to fill the Office of Drinking Water's data gaps and to develop the concept of surrogate measures for estimating the toxicity of complex mixtures.

2.3.3.4 Biological Markers

Analytical methods (^{32}P-postlabeling and cytogenetic) will be improved for increased sensitivity and resolution, and these will be validated for both biomonitoring and site evaluation. Exposure, dose, and cytogenetic effects relationships in blood lymphocytes of rodents and humans exposed to chemicals will be determined. These methods and calibration studies will provide the experimental background for measuring DNA adducts and the cytogenetic effects induced in humans environmentally exposed to these substances.

Biomarkers that can distinguish preneoplastic or neoplastic cells in human or experimental animal carcinogenesis will be developed and validated. The occurrence and frequency of alterations of genes implicated in the cancer process will be assessed in tissues and cell types that are relevant to environmental cancer etiology. In particular, carcinogen-exposed cells from humans and from rodents will be compared for changes in expression of specific cellular oncogenes (the *ras* and *myc* family), alterations in gene copy number, and the presence of specific DNA sequence alterations. In addition, other markers of cell differentiation or functions, such as receptors that code for essential nutrients or factors, will be measured in human tissues that have been cultured or by

culture of animal tissues. Analysis of DNA sequence alterations will be performed, and alterations in genes or their expression will be analyzed. This research involves studies on the genetic effects induced by environmental carcinogens at specific sites in target genes using a shuttle vector system. Relationships between identified biomarkers, genetic effects, and carcinogen exposure will be studied with regard to mechanistic consistency.

GTD has developed the capability to conduct integrated exposure assessment studies jointly with the monitoring and engineering laboratories. Future research in this area will focus on improving methods to conduct total human exposure assessments that employ new genetic bioassays for biomonitoring external exposure and biomarkers for internal exposure assessment (DNA and protein adducts and metabolites). In studies jointly conducted with the Human Studies Division, human tissues and body fluids will be used to assess the relationships between internal exposure, biologically effective dose (target tissue dose), and genotoxic and nongenotoxic effects (e.g., cytogenetic effects).

2.3.3.5 Pollutant Mixtures

Genetic bioassay methods will be developed in conjunction with personal and microenvironmental sampling methods in order to use bioassays in the biomonitoring of exposure to complex mixtures. Research in the water and waste areas will focus on developing alternative bioassays (*in vitro*, cellular systems or fish and plant systems) for monitoring industrial effluents, waste sites, and drinking water.

Complex-mixture risk extrapolation issues are increasingly emerging in all areas of air toxics (e.g., urban soup, alternative fuels) and in indoor air research. Future GTD air research will expand the use of new methodologies for identifying and assessing complex mixtures of air pollutants. These methodologies will encompass human and environmental exposure assessment through genetic bioassays for both external and internal exposure assessment. New emphasis will be applied to source apportionment of exposure sources; future research efforts will apply such methodologies to complex mixtures of DNA adducts from human exposures. The comparative potency and parallelogram methodologies will be further expanded and applied to new air risk problems; at the same time, they will be used to test additivity assumptions that may be used in alternative approaches.

The current risk assessment guidelines that employ additivity are most widely used in conjunction with Superfund site evaluations and in the assessment of waste incineration because very little toxicology data are available for the complex waste mixtures to which humans are exposed. As more hazardous municipal and hospital waste is incinerated rather than buried, toxicology data on the complex mixture of incineration emissions will become increasingly critical; and GTD research efforts will continue to be directed to developing the methods and database necessary for the cancer risk assessment of these emissions. Evaluating the gaseous emissions will become more important in the future, and GTD researchers will develop and employ new direct bioassay methods for this task that analyze the emissions both before and after atmospheric transformation. Incineration emission research, as well as the additivity work mentioned above, will focus on chemical class interactions. Potential interactions between chlorinated dioxins, metals, PAH, and substituted PAH are important subjects for these efforts.

2.3.4 Emerging Issues

2.3.4.1 Nongenotoxic Cancer

Work over the past 30 years has uncovered a variety of mechanisms of action for carcinogens, including ones that involve the induction of cancer by direct (genotoxic) or indirect (nongenotoxic) interactions with the genome. The incorporation of mechanisms of action into risk assessment is a key element of that process. When positive correlations are made between the mechanisms responsible for an agent's known biological effects and the currently understood mechanisms of carcinogenesis, mechanistic information can be used with more confidence in determining the hazard to man.

Although a good deal of information has been gathered on the mechanisms of action of genotoxic carcinogens, data on nongenotoxic carcinogens are sparse. Nongenotoxic carcinogens are thought to have a wide range of mechanisms of action because this class of chemicals is so heterogeneous (e.g., tumor promoters, peroxisome proliferators, solid state carcinogens, hormones, and hyperplastic agents). Studies are needed to identify these mechanisms in experimental animals and in man through exposure of rodent and human mesenchymal or epithelial cells. The effects of this exposure to nongenotoxic chemicals should be measured at the molecular level in terms of alterations in genes and gene products thought to be associated with the induction or progression of cancer.

2.3.4.2 Susceptible Populations

The risk assessment process both for cancer and for heritable gene mutation is based on extrapolations from available animal data. The quantitated risk factor is generally calculated for the "average" human even though the human population consists of a heterogenous mix of diverse genetic backgrounds. This genetic diversity, coupled with environmentally induced illness or ill health (infectious or chemically induced, such as cigarette smoking), creates a stratified population with varying susceptibility to particular environmental exposures. As our ability to quantitate the risk factor for the "normal" human improves, the need to determine and evaluate the risk for susceptible subpopulations will emerge as a major research area.

Identifying populations of individuals with unusual sensitivity to certain cancers fosters understanding of the etiology and mechanism of occurrence of those cancers. A major issue in cancer mechanism concerns the mechanistic role of oncogene alteration and expression in human cancer and the oncogene's role as a biomarker. Work in this area will benefit by significant interaction with other investigations on "molecular mechanisms of carcinogenesis." The combined research effort is aimed at determining the role of environmental chemicals in the expression of oncogenes and tumor suppressor genes. By establishing the biological basis for the alteration of these genes, the research effort can also assess the usefulness of these genes as biomarkers for preneoplastic and neoplastic lesions in exposed or susceptible human populations.

2.3.4.3 Microorganisms

The health effects caused by microorganisms will be an important concern for EPA in the coming years. Significant research issues associated with this problem include:

- Potential adverse health effects due to the registration of engineered organisms
- Potential adverse health effects due to the use of microorganisms for environmental bioremediation
- Understanding the role microorganisms play in the "sick building syndrome"
- Potential release of pathogenic organisms, especially viruses, during the incineration of medical/pathological waste

The first two of these issues are addressed to some extent in the Biotechnology Health Research Strategy Plan. For example, a BSAC subcommittee has recommended that EPA examine the interaction of pollutants"especially metals"and the co-selection of antibiotic resistant microorganisms. Due to increased recirculation of air within buildings and lower air exchange rates due to insulation, an increasing number of "sick building" syndromes are being caused by organisms that are not often seen in clinical settings (e.g., aspergillus). To separate chemically introduced causes of adverse health effects from those that are induced by microorganisms or interactive, EPA should establish a program that encompasses health work associated with such organisms as fungi, soil bacteria, and spirochetes.

Finally, most medical facilities are now turning to incineration (municipal and facility) to dispose of biological, medical/pathological waste. Some incinerator conditions (e.g., malfunctions that cause bursts of unburnt components to be emitted) allow pathogenic organisms to be encountered in either the airborne or ash waste streams.

2.3.5 Summary

2.3.5.1 Hazard Identification

GTD will increase efforts in computational structure-activity analysis using quantitative mutagenesis, carcinogenesis, and developmental toxicological data. Test method development efforts will address those chemical classes (e.g., chlorinated compounds) and kinds of genetic damage (e.g., aneuploidy) that are not readily detected using present techniques. Continued mechanistic studies on mutation in germ cells will lead to the development of improved analytical capabilities to detect such changes. Methods to detect chemicals that induce tumors through nongenotoxic mechanisms also will be given special attention.

To identify potential environmental health hazards of special programmatic interest, researchers will focus on biological markers of dose and effect, *in situ* bioassay methods, and microbiological investigations on recombinant microorganisms.

2.3.5.2 Dose-Response Assessment

Mechanistic studies will form the cornerstone of research in carcinogenesis and heritable mutagenesis. The development and ultimately the usefulness of biologically

based dose-response models depend on the recognition that carcinogenesis and heritable mutagenesis are both complex, multistaged biological processes. Since genetic changes are clearly involved in the etiology of cancer, the development of a set of mechanistically consistent biomarkers of genetic change may be expected to lead to a clearer understanding of both mutagenesis and carcinogenesis in experimental animals and in man. Particular attention will be paid to developing models that improve the ability to extrapolate from rodents to humans.

A mechanistic focus on the biological basis for altered gene expression should yield techniques that will 1) demonstrate the importance of oncogenes and tumor suppressor genes in carcinogenesis, 2) shorten the time for analysis of the mutagenic and carcinogenic activity of chemicals in humans, and 3) identify populations of individuals with unusual sensitivity to certain genetic diseases and cancers.

GTD's complex mixture research will emphasize improved methodology for the application of bioassay and molecular genetic methods in the identification of mutagens and carcinogens. Researchers will make increased effort to interpret and extrapolate the results from comparative biological evaluations in human, animal, and *in vitro* studies of various types of complex mixtures. Research on component interactions and modeling assumptions (e.g., additivity, antagonism, synergism) will be given special attention in order to address complex mixture risk assessment issues.

2.4 ENVIRONMENTAL TOXICOLOGY DIVISION

Humans are exposed to thousands of toxic chemicals by inhalation, ingestion, and dermal contact. The surfaces of the respiratory tract, gastrointestinal (G1) tract, and skin function as primary barriers, or portals of entry, that separate viscera from toxic chemicals; however, chemicals can cross these physical barriers by both active and passive processes of absorption and enter the bloodstream. During absorption, the chemicals can also be transformed. The major portals of entry for chemicals are the:

- Skin
- Lung
- GI tract

Introduction of the chemicals into the body can result in cellular damage, disease, changes in metabolic processes, or activation of protective mechanisms. Moreover, because the functional integrity of the organs acting as protective barriers may be compromised by their defensive roles, susceptible populations"such as children, the elderly, and people with chronic lung or liver disease"can be at risk of disease with only minor chemical challenge.

Obstructive diseases of the lungs (i.e., emphysema, asthma, chronic bronchitis) are the third leading cause of death among the populace. While cigarette smoke accounts for a considerable portion of the 50,000 deaths per year, deaths due to obstructive lung diseases are increasing. Morbidity, which is more closely associated with the overall health of the population and the economy, follows a similar, but more substantial course in terms of person-years lost. Ozone and smog-related pollutants have been associated with the respiratory infections that strike 80-100,000 people per year and kill more than 60,000 per year. Similarly, exposure to anthropogenic pollutants or disinfectant-derived *de novo* pollutants in water has been associated with neoplasms and cardiovascular diseases that kill or disable millions each year.

Environmentally linked diseases encompass an extremely broad range of human illnesses. Examples include bronchitis and/or emphysema in persons chronically exposed to fossil fuel-derived air pollution, lung fibrosis and cancer in individuals exposed to asbestos, heart and vascular diseases in individuals exposed to carbon monoxide, and impairment of immune function in men and women exposed to certain pesticides. In addition, the possible effects of chronic exposure to cigarette smoke are well documented, including lung and other cancers, heart disease, and respiratory diseases. Less severe effects have been tentatively attributed to passive inhalation of cigarette smoke in chronically exposed nonsmokers, and recent evidence suggests that children are a sensitive group for this pollutant.

The immune system is both a target for and a defense against environmental insult. For example, suppression of a delayed hypersensitivity response and increased susceptibility to respiratory infections have been found in patients who accidentally ingested pesticide-contaminated rice oil. Although the long-term deleterious consequences of exposure to polybrominated biphenyls are as yet undocumented in humans, early data indicate a correlation between immune alterations and increased tumor incidence. Other pollutants such as nitrogen dioxide may alter immune function and increase the risk of respiratory infections, most notably of viruses. Because a large num-

ber of people die from respiratory infections each year, even a small effect on immune system components or their functions can have major consequences on the physical and economic health of the population.

Pharmacokinetics, which defines the absorption, distribution, metabolism, and excretion rates of environmental chemicals, can be used to highlight the relationship between exposure and effect. Body mechanisms and processes, such as deposition and clearance of inhaled particles, affect the amount of the chemical that is absorbed rather than eliminated and that reaches a target tissue or active site (e.g., cell membrane, receptor, gene) in the form of a parent compound, a form that was absorbed or distributed to that site, or a metabolite. This difference between exposure and dose concentrations affects the severity of the health effects that potentially result from exposure to the chemical and complicates extrapolations from one set of conditions to another (e.g., route-to-route, high-to-low-dose, acute-to-chronic-exposure, species-to-species). Confidence in these extrapolations can be increased by both experimental and modeling dosimetric studies.

2.4.1 Divisional Program

The Environmental Toxicology Division (ETD) uses both animal research and *in vitro* methodologies to study the potential for human health effects associated with exposure to environmental pollutants. Research efforts will cover exposure, emphasizing the three primary routes by which the environmental pollutants enter the body (inhalation, oral, and dermal); dose, in an effort to describe and predict the concentration of pollutant that reaches the target organ(s); and effects, focusing on the response in the respiratory and immune systems, with expertise developed as needed in the cardiovascular, hepatic, renal, dermal, and gastrointestinal systems. The division's research efforts in the three areas of the risk assessment paradigm are focused on improving human health risk assessment through quantitative animal-to-human extrapolations (see Figure 2-6).

ETD's research program is unique among the HERL divisions in that it comprises many areas of biological expertise and encompasses a broad spectrum of environmental health research. The division serves as a primary technical resource within the Agency for activities requiring expertise in animal inhalation studies and in the health effects of air pollutants. Because of the diversity of scientific approaches to the research issues, multidisciplinary teams composed of scientists inside and outside the division are vital for the success of ETD research efforts.

2.4.2 Division-Specific Research Needs

2.4.2.1 Hazard Identification

One of the most critical research issues facing EPA is identifying potential health hazards so that the relationship between environmental exposures and health effects can be predicted and interpreted. To meet this end, the Agency needs validated, short-term test methods to screen new and existing environmental threats. The tests should allow for timely determination of causality and adversity.

Similarly, animal models of infectious/neoplastic and allergic disease need to be developed that correlate the effects of toxic chemicals on immune function tests and thus improve estimates of the risk of increased disease. As an example, effects of several toxic chemicals administered by several routes on natural killer cell activity in mice (a commonly used immune function test) have been correlated with effects on susceptibility to mouse cytomegalovirus (a common opportunistic infection). Hence, chemicals that suppress this particular immune function test can be predicted to also increase susceptibility to certain types of infection.

2.4.2.2 Dose-Response Assessment

Dose-response assessment comprises three areas of research: 1) the relationship between exposure and dose, 2) the relationship between dose and biological response, and 3) the biological mechanism responsible for the observed effect. ETD can identify specific research needs in all three areas. To understand the relationship between exposure and dose to the target tissue, both experimental and theoretical approaches must be used to define dosimetric and PB-PK models of inhaled, ingested, and dermally applied chemicals. Because of the importance of the lung, skin, and gastrointestinal tract as both target organs and the major portals of entry for pollutants, research into the uptake and absorption of pollutant gases, solvents, and particles is critical.

A wide variety of chemical and physical factors, depending on the portal of entry, determine uptake and absorption rates. Data are needed, for example, on the following factors:

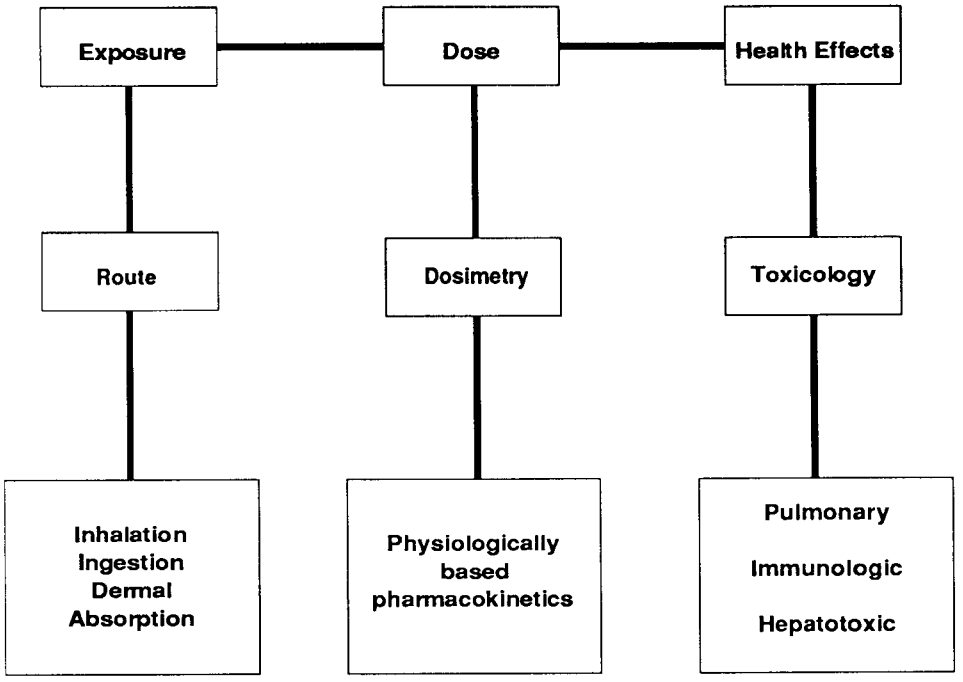

Fig. 2-6:
Environmental Toxicology Division Research Perspective

- Determinants of gaseous and particle deposition in and clearance from the respiratory tract, including morphology

- Experimental measurements of physiological factors governing chemical disposition

- Comparison of *in vivo* and *in vitro* absorption and metabolism systems

- Rates of chemical reactions between pollutants and the liquid linings and tissues of the portals of entry; determining these requires a detailed knowledge of the particular biochemical constituents within liquid linings and tissue

- Morphology of the portals of entry at the macroscopic and microscopic levels

To conduct reliable and cost-effective extrapolation between animals and humans and between routes of exposure, researchers must account for all of these factors in the mathematical models they develop.

The next step in dose-response assessment is to explore the relationship between target organ dose and biological response by developing animal models of human disease states (e.g., cardiopulmonary disease). Animal models of various cardiopulmonary disease states such as emphysema, asthma, and chronic obstructive and restrictive lung diseases are critical to understanding the development and progression of lung dysfunction and pathology in humans, especially under conditions of chronic exposure. For example, the role of endogenously released elastase from inflammatory cells is now known to be a key element in the development of emphysema. Exogenously administered elastase to the animal lung accelerates a pathogenesis that closely resembles a state of illness which takes decades in man. The development of this animal model has revealed that α1-antitrypsin (α1-Pi) also plays an important role in the pathogenesis of emphysema, since it normally inhibits secreted elastases. These findings demonstrate that the same disease entity can be induced by quite different mechanisms"induction of inflammation or inhibition of α1-Pi. Hence, modeling is critical for understanding not only the pathogenesis of disease, but also the impact of environmental stresses that can induce or enhance its progression.

A critical research need lies in determining deposition and clearance patterns of inhaled compounds. From a dosimetric point of view, the lung is currently considered as a whole. Inhaled particle dose is computed assuming 100 percent deposition efficiency, no clearance, and no distinction among different regions of the lung. This approach ignores the dynamics of deposition and clearance as well as the nonhomogeneity of various regions, both in structure and function and in response to inhaled pollutants. To better relate response to dose of inhaled particles, the regional deposition and clearance of particles must be quantitated in animals and correlated with similar data in humans.

Another area of needed research is the mechanistic origin of cardiovascular, pulmonary, liver, and immune function disease. Work in this area is critical for ex-

trapolations across species, from acute to chronic exposures, and from high to low doses. Two related issues are species sensitivity and animal-to-human extrapolations, including route-to-route, acute-to-chronic, and *in vitro*-to-*in vivo*. Research needs associated with these issues center on gathering experimental data across species that will fill the theoretical data gaps for animal-to-human extrapolations. Several approaches are possible:

- *In vivo* monitoring of the deposition and clearance of radiolabeled particles in animals
- Investigating the relationship between macrophage functions and particle clearance
- Microscopic analysis of tissue samples to assess regional dose and effect

2.4.2.3 Chemical-Specific Data

Some of EPA's specific short-term research needs include information specific to certain chemicals, such as:

- High-production chemicals
- National Ambient Air Quality Standards (NAAQS) pollutants (e.g., subchronic and chronic O_3, NO_2, methanol, and acid aerosols)

2.4.2.4 Pollutant Mixtures

EPA research needs also include information on:

- Specific complex mixtures (e.g., alternative fuels, urban mixtures, drinking water disinfectants, and indoor air mixtures)

EPA must develop strategies to handle issues of potentially chronic noncancer health effects and their relationship to acute response and interactions of atmospheric mixtures. In conjunction with the Human Studies Division (HSD), ETD conducts chemical-specific studies using similar research protocols to improve animal-to-human extrapolations.

2.4.3 Research Plan

2.4.3.1 Hazard Identification

ETD work under this research topic presently includes:

- Development of immunotoxicologic methods in support of immunotoxicity guidelines
- Validation and refinement of methodologies to improve predictive capabilities in pulmonary toxicology, immunotoxicology, and other target organ toxicities
- Physiologically based pharmacokinetic (PB-PK) model development based on experimental data

ETD will continue to develop an immunotoxicity testing tier in rats similar to that for mice by 1) developing appropriate host resistance models in rats, and 2) adapting immune function tests to rats for purposes of supporting guideline development. In addition to the traditional testing schemes analogous to those applied in mice, methods for assessing immune responses in the lung and host resistance models that are particularly applicable to the assessment of effects from inhaled compounds will be developed. A major goal of these research efforts will be to correlate effects of chemical exposure on immune function tests with effects on susceptibility to infectious, neoplastic allergic or autoimmune disease; host resistance models will be used to study the adversity of alterations in immune function.

Work on PB-PK models comprises a major component of the SAR research effort. Such research is critical to hazard identification because SAR approaches to both toxicology and dosimetry may allow estimates of risk for compounds without the need for detailed toxicity testing or pharmacokinetic determinations. For example, a compound (so long as it is not highly toxic) may be excluded as a dermal exposure hazard because its chemical structure leads to minimal dermal penetration.

2.4.3.2 Dose-Response Assessment

To perform accurate quantitative animal-to-human extrapolations, researchers must lessen the uncertainty associated with extrapolating effects associated with one route of exposure to those associated with another. To aid in this task, ETD is investigating, both experimentally and theoretically, the absorption of toxic chemicals by all three

primary routes of exposure. The different processes that affect the quantity of chemical absorbed through a particular route (e.g., similarity or dissimilarity of partitioning and absorption across membrane barriers) are major concerns. These studies require expertise in inhalation engineering, inhalation toxicology, systemic and *in vitro* toxicology, dermal absorption, pharmacokinetics, and metabolism.

ETD also addresses issues such as gas and particle dosimetry and extrapolation accuracy. Research efforts are directed to developing and validating biomathematical models to compare intra- and interspecies dosimetry data. ETD is developing models for the primary routes of exposure that can accurately predict the temporal distribution of toxicants in various organ systems. Such models are of particular use in quantitatively extrapolating effective pollutant concentrations between animals and man. Other work is focused on development of state-of-the-art modeling techniques for route-to-route, acute-to-chronic, *in vitro*-to-*in vivo*, and animal-to-man extrapolations. Physiologically based dosimetry models and structure-activity principles form the basis for theoretical constructs to examine dose-effect relationships.

PB-PK research involves extensive work in both experimental research and modeling. Once a compound penetrates beyond the portal of entry, it enters the systemic circulation and can be taken up by the systemic organs, such as the liver and kidney. Predicting the amount of a compound that will appear in an organ as a function of time can be addressed through PB-PK modeling. The data these models require as input include organ size, blood flow, blood-tissue partition coefficients, and metabolism rates. Because these data have usually not been gathered for compounds of interest to the Agency, further experiments are required. Established methodologies can be used to obtain some of these data, but gathering some of the input data will require development of new methodologies. To be useful, PB-PK models must not be compound- or species-specific; in other words, they must allow highly accurate predictions of dose with only minimal input. Further knowledge about structure-activity relationships (SAR) will help achieve this goal.

ETD researchers will also develop and use the analytical methods that are essential for quantifying the internal dose of a xenobiotic and its critical metabolites per unit of sensitive (target) tissues or cells. Such data are used in biomathematical models for making intra- and interspecies comparisons of dose-response relationships in animals and humans in terms of tissue levels of key toxicologic substances. This conversion is dependent on a thorough knowledge of the metabolism and pharmacokinetics of the parent chemical in the species of interest. Experimental efforts include both *in vitro* and *in vivo* methodologies for the determination of metabolic rate constants and the validation of extrapolation from one database to another. Methods for the isolation and identification of chemicals and metabolites in tissues and biological fluids are being developed and applied in experimental dosimetry research programs that address various types of extrapolation. ETD's pharmacokinetic and toxicodynamic research addresses problems relevant to all decision units that provide resources to HERL.

In addition, ETD investigates the toxic health effects of environmental pollutants using laboratory animals. So that this research can be used more directly in regulatory processes, a major effort using animal models of potentially impaired humans is underway to determine whether certain segments of the population are more susceptible to air pollutants. This work includes the use of conventional techniques as well as the development of new methods to evaluate the effects of pollutants on the lung, skin, liver, immune, host defense, and cardiovascular systems. Although pulmonary effects and route of exposure comprise a major focus for much of the animal research, additional emphasis will be directed toward the dermal and oral routes in order to better assess the health consequences of environmental exposures.

To help identify adverse health effects, ETD researchers will work on correlating structural, biochemical, and functional changes in animal organ systems, principally the lung and liver. Such correlation is inherently complex, requiring a research team with specialists in biostatistics, biochemistry, pharmacology, engineering, immunology, physiology, physics, toxicology, and chemistry. As pharmacokinetic profiles are developed for xenobiotics, ETD will continue to study the biochemical, physiologic, and pathologic effects of these substances to ascertain mechanisms of toxic action. Specific health effects issues being addressed include animal-to-man relationships in toxic response, mechanisms of injury, animal models of disease, chronic lung disease, and species sensitivity.

ETD studies on the chronic effects of environmental pollutants cross a variety of scientific disciplines, including inhalation engineering, biostatistics, cardiopulmonary physiology, biochemistry, immunology, morphometry, and histopathology. A primary focus of ETD research has been the lung; thus, the design, fabrication, and operation of state-of-the-art inhalation facilities by the ETD engineering staff is essential. Such engineering-based re-

search and development that meets the exposure requests for a spectrum of gases, particles, and mixtures is an ongoing function of a broadly skilled team experienced with both acute and chronic exposures of laboratory animals to a wide variety of gases and particles.

Given the myriad of chemicals in air, it is critical that ETD investigate the effects of inhalation exposure to these pollutants on pulmonary and cardiovascular function. Hazard identification is the first step; but, as data from the primary air pollutants show, certain subpopulations are likely to be more sensitive than others to the adverse effects of toxic inhalants. Hence, emphasis must be placed on the development and use of animal models that resemble human disease states such as emphysema, asthma, hypertension, congestive heart failure, and pulmonary fibrosis. Assessment of the relative sensitivity of these subgroups as compared to the normal healthy state would also provide basic susceptibility data as well as information on the mechanisms of response that come into play in the responses seen in the normal, less susceptible state.

At present, most animals used in experimental studies are highly inbred strains. While these animals provide good control of that experimental variable, perturbing the animals' health through selective/specific empirical techniques is useful for inducing potential risk factors to the target organ of interest"the lung, for example. Given sufficient knowledge of a particular disease state or the mechanisms by which the risk factor is induced, a disease state can be induced. No intervention (chemical, surgical, or otherwise) is totally specific; thus, this imposed factor must be incorporated into all data interpretation.

This disadvantage may soon be ameliorated by new research in genetic manipulation. Human genes can now be transplanted into the embryos of newly developed, genetically altered rodent strains to "create" so-called syngeneic animals; this work has opened a new avenue for this type of research. The advantages of exploring the genetic basis for a disease"e.g., specificity, control"may greatly aid in health interpretations. Using these new syngeneic animals as well as refined, (typically) chemically induced models of disease, ETD researchers will elucidate alterations in pathogenesis, overall health sensitivities, and mechanisms of action. Studies such as these should provide great insight into those abnormal or deficient states of health that are most likely to be worsened by exposure to environmental pollutants, or that could subject the whole organism to these effects.

ETD investigation of the effects of environmental chemicals on immune functions and on a variety of disease models will have three major goals:

- Developing methods for assessing immune responses in the lung to improve the assessment of the immunotoxic effects of inhaled compounds; these methods could then be used along with host resistance models to provide information on chemicals regulated by NAAQS and other air toxic compounds for regulatory purposes

- Correlating effects of toxic chemicals on immune function tests with effects on host resistance and disease models to improve the assessment of risk of increased debility based on effects of immune function tests

- Improving predictions of the effects on humans based on animal research

A key aspect of ETD's research program is its ability to relate exposure to dose to effect in a coordinated manner. Dosimetric models of inhaled compounds require inputs of measurable physical and chemical parameters. Once models are developed, they will be validated experimentally and further refined. A coordinated research approach involving modelers and experimentalists is essential for successful development, validation, and refinement.

Once exposure concentration of a pollutant can be related to tissue dose through dosimetric techniques, research on relative tissue sensitivity among species can be conducted. Hence, in concert with dosimetry research, animal toxicology research will focus on the issues of homology and species sensitivity of experimental laboratory animals relative to humans. Identification of acute responses to inhaled toxicants and the responsible underlying mechanisms over relevant dose ranges will be key to understanding the relevance of the particular animal model to humans. For example, a more thorough understanding of inflammatory processes in the lung and the events leading to tissue injury or disease will enable a better comparison with the fragmented human data and establish a base from which an assessment of chronic health risks can be derived (only animals studies can provide controlled chronic exposure studies to ascertain long-term effects). Identification of factors that lead to disease or otherwise contribute to susceptibility of the exposed subpopulations is an important step in evaluating the major air toxicants in the urban environment. The collective ef-

forts from a multidisciplined group of investigators"from ETD, HSD, and collaborating universities"will develop better study models and potentially useful markers or indicators of risk for use in human populations.

The appropriateness of any animal model used in the study of a toxicological event is most clear when the response of the animal can be correlated, at least qualitatively, with the human response (i.e., a homology of effect exists). If homology exists, then determination of relative dosimetry and sensitivity will provide a straightforward picture of the phenomenon that can be directly incorporated into the risk assessment process. Unfortunately, because this information scenario is not typical, the mechanism of injury must be elucidated for toxicological data to be used confidently (i.e., with minimal uncertainty) in risk assessment. By such a process, the type and degree of injury can be better related to the human situation. ETD investigations in this area will amplify understanding of the mechanisms of toxicity; additionally, they will enhance interpretations of the chronic toxicity data for which there is virtually no human exposure correlation.

The importance of the various routes of exposure is clearly associated with the physical form of the pollutant in the environment, although all exposures occur by a mixture of routes. Water pollutants enter the body primarily through ingestion; ambient air pollutants (both gases and particles) enter primarily through the lung, though dermal absorption is important for exposure to compounds such as pesticides. Despite such distinctions, a potentially considerable overlap exists between exposure routes. The portal of entry by which a pollutant enters the body can have a significant impact on the dose to the eventual target organ"especially if the portal of entry is also the primary target organ.

When a pollutant, or class of pollutants, can enter the body by multiple routes, it is both costly and impractical to conduct toxicity testing by all routes of exposure. Because the portal of entry can influence eventual organ dose, however, the results of a toxicity test by one exposure route must be extrapolated to predict the outcome of exposure by another route. The level of research effort into portal-of-entry questions is determined 1) by the complexity of the organ serving as the portal of entry, and 2) by the organ's accessibility to direct experimentation. Research into gas and particle uptake by the respiratory tract requires a high level of effort because of the complex physical interactions that occur, and because of the wide diversity of cellular and biochemical components in this organ system. These problems are further complicated by the inaccessibility of the lung to direct observation in intact animals.

In contrast, dermal absorption of compounds can be measured directly, because the skin is a readily accessible organ to experimentation. Studying gastrointestinal absorption of compounds is a problem midway in difficulty between work on the lung and skin. The GI tract lends itself to more simple compartmental modeling approaches than the lung, but it still requires more coordinated experimental and modeling approaches than the skin.

Many proteins that are important to cell functioning are lipophilic and resident in the cell membrane. The lipid bilayer that forms the membrane provides a matrix in which the proteins may move relative to one another. Some functions are dependent on two proteins being able to diffuse into proximity with one another. The rate at which a protein can move in the lipid matrix is limited by the fluidity of the membrane, a characteristic that is the inverse of viscosity. The functions of several membrane proteins are known to be affected by the fluidity of the membrane. In particular, fluidity affects the binding of neurotransmitters for some receptors, e.g., the serotonin and the beta-adrenergic receptors.

Most organic xenobiotics are lipophilic and rapidly partition into cell membranes, with a steady-state concentration in the cell being reached with continuous exposure. Accumulation of the compound in the membrane will affect its fluidity and consequently the function of the membrane proteins that are sensitive to fluidity. Many anesthetics work in this manner, although the detailed mechanism is not well understood. Xenobiotics that are not metabolized or only slowly metabolized could also act in this manner, and may result in toxicity at the cellular level. This response will be investigated by examining receptor binding as a function of fluidity (i.e., concentration of chemical) induced by potentially toxic chemicals representing different structural classes. This research effort is designed to examine the role of membrane properties in toxicity, explain the basis for toxic effects, and identify heretofore unsuspected toxicants.

The rat has become a primary animal model used in toxicological research; however, this rodent species may not be the most appropriate system in which to study all types of responses. Differences in metabolism or antioxidant levels between the rodent and human, for example, could result in divergent responses that may affect either the homology or sensitivity of effect. The mouse offers another rodent model with extensive genetic charac-

terization. In fact, the mouse is frequently the species of choice for certain mechanistic studies. The animal model should be chosen in light of the specific questions under study. Various responses should be studied in at least one other species; such data, when used in conjunction with the limited human data, would allow researchers to piece the puzzle of response together with relevant dosimetry to determine ultimate human risk. This task is not simple, but well-conceived studies of response in multiple species and dosimetry will yield more useful health assessments.

For example, guinea pigs have a very sensitive bronchoconstrictive reflex to irritants such as sulfuric acid, and rats do not. On the other hand, long-term exposures to acid in rats induces a pathology resembling bronchitis in man, but similar exposures do not appear to affect guinea pigs to the same degree. Such a disparity is difficult to interpret: perhaps dosimetric as well as species-specific factors such as mucus fluid neutralization capacities are involved. Such differences in response should guide investigators in selecting appropriate species as well as in interpreting data for comparison to humans.

Clearance of inhaled nonaqueous particles from the respiratory tract of animals is another focus of ETD research. The database on clearance of particles from the conducting airways and pulmonary regions of animal lungs is very sparse. Yet, clearance kinetics is an important component, along with deposition, of particle dosimetry models. ETD investigations, conducted in concert with HSD, will focus on whole lung kinetics as well as on the cellular processes that govern clearance kinetics.

Toxicokinetics of environmental chemicals will become a major focus of ETD research. The absorption of compounds by the oral, dermal, and inhalation routes of exposure will be studied both *in vivo* and with *in vitro* methodology. Researchers will examine *in vivo* the distribution of the absorbed compound and develop models to predict such transport. They will also analyze the rate of metabolism in the liver, portals of entry, and target tissue in the animal as well as in tissue extracts. Excretion of both the parent and metabolite compounds will be studied, with emphasis on pulmonary elimination of volatiles, hepatobiliary elimination of large molecules, and renal elimination of the majority of chemicals. *In vitro* methodologies will be developed and used where appropriate, so that researchers can measure the integrated dose of toxic chemical to the target tissue for representatives of a given class of chemicals. Physiological and experimental data will be used as input for pharmacokinetic models, which will be designed to allow dose, route, and species comparisons.

2.4.3.3 Chemical-Specific Data

ETD must continue to work on the toxicities of specific toxicants to assist the program offices in developing emission and exposure standards for the myriad of chemicals in the environment. At present, these chemicals are controlled individually; but so little is known about most of them that developing control strategies for interactions is impossible without sufficient databases. ETD research is progressing on several levels:

- Identifying health effects
- Assessing the chemicals' dose-response behaviors
- Testing the worthiness of dosimetric and PB-PK models in predicting observations (so that data can be extrapolated across species to humans)

Interactions that may include metabolism, while under investigation as single and mixed toxicants, will provide a new dimension to this effort; and, as a result of this work, the ever-evolving tools for risk assessment should be improved in their capacity to estimate potential human hazards. ETD research is designed to integrate components from outdoor and indoor issues because these comprise the entire exposure scenario. Much is unknown about the effects of encountered chemicals in their particular exposure scenario"for example, volatile organic compounds (VOCs) found indoors or alternative fuels adding to urban "soup."

The health issues facing experimental toxicology are growing more complex and urgent. Current regulations based on acute alterations in lung function in exposed humans have little relevance for the potential threat of chronic lung disease or impairment. Moreover, single-contaminant studies clearly oversimplify even the classic atmospheres of cities like Los Angeles.

Recent decisions to replace conventional fuels with alternative, more combustible fuels are intended to reduce tropospheric ozone as well as national dependence on foreign oil. The enhanced volatility of these alternative fuels and their combustion products, however, may lead to additional atmospheric exposures. Possible health effects associated with the switch require examination.

2.4.3.4 Biological Markers

In collaboration with HSD, ETD will conduct research to develop and evaluate quantitative biochemical markers for toxic chemical exposure, and to integrate these markers into current models of toxicity, adaptation, and repair. In this effort, relationships between tissue dose (binding or absorption of toxicant molecules) and toxicity will be examined, as well as between species, exposure scenarios, and altered states of susceptibility. The linkages developed through this research should address EPA's need to extrapolate data from animals to humans, from acute to chronic situations, and from resistant to susceptible individuals. Questions involving the adversity of biochemical changes will be addressed through correlation of these changes with morphological and physiological effects. Research efforts will also include development of immunologic assays as biomarkers for allergenic reactions to inhaled compounds and establishment of methods for testing the allergenic potential of inhaled compounds.

2.4.3.5 Pollutant Mixtures

Much ETD research on complex mixtures is covered under Section 2.4.3.3, Chemical-Specific Data. In addition, ETD plans to develop bioassays to evaluate the toxicity of complex mixtures.

2.4.4 Emerging Issues

2.4.4.1 Biologicals/Microorganisms

In spite of our substantial knowledge of microbiology, the role of microorganisms and their products as one component of the environmental milieu is not well appreciated. Clearly, Legionnaire's disease is a salient example of the insidious influence of microorganisms on health, and the polio epidemic of the 1940s and 50s stands out as a waterborne problem. Today, we are just beginning to consider the many microorganisms that populate our homes and work places; and data concerning cases of sick building syndrome suggest that these life forms, their spores, and gaseous emanations may contribute to impaired health"from malaise to respiratory stress.

Destruction of biological wastes, particularly from medical applications, is another cause of concern. Incineration is probably the most efficient, but expensive, means of destruction and decontamination. If these incinerators are poorly operated, the contaminated materials may not be totally destroyed and thus the smoke from the incinerators may be contaminated. The resolution seems easy enough, but the problem is of growing concern due to lack of knowledge and enforcement.

2.4.4.2 Natural Versus Synthetic Fibers

The health effects associated with asbestos, a naturally occurring fiber, are well known. As manmade fibers are developed, questions arise as to their potential for similar health consequences. Research into the dosimetry and the potential for a relationship between fiber dosimetry and toxicity needs to be explored.

2.4.4.3 Ultraviolet Radiation

Sunlight contains an ultraviolet light of short wave length known as the "B form" that appears to have not only genetic implications (skin carcinogenesis), but may penetrate enough to affect systemic immune function. Too little is known at this time to do anything but speculate on the problem. If UV-B has immune suppressive capabilities, however, any increases in this type of radiation due to ozone layer degradation could represent a problem of potentially great significance.

2.4.4.4 Bioavailability of Chemicals from Soils or Other Matrices

Many potentially toxic compounds are found in the soil and may pose a hazard to humans by all three routes of exposure. Because these compounds may be bound up in the soil matrix, their potential health consequences are likely to be a function of the ease with which they can be released from the soil after it has entered the body through the gut or respiratory tract, or after it is applied to the skin. Research into this issue is likely to be important for future assessment of hazards from toxic waste sites.

2.4.5 Summary

2.4.5.1 Hazard Identification

Because more cellular and molecular approaches, in addition to pulmonary function tests, are needed to identify hazards, researchers will develop *in vitro* methodologies for evaluating pulmonary toxicity. Mechanistic studies on respiratory injury will focus on the pathogenesis of toxicant-induced lung disorders, with emphasis on the development of animal models for pulmonary disease and populations at special risk (e.g., children, the elderly). In all cases, studies will be con-

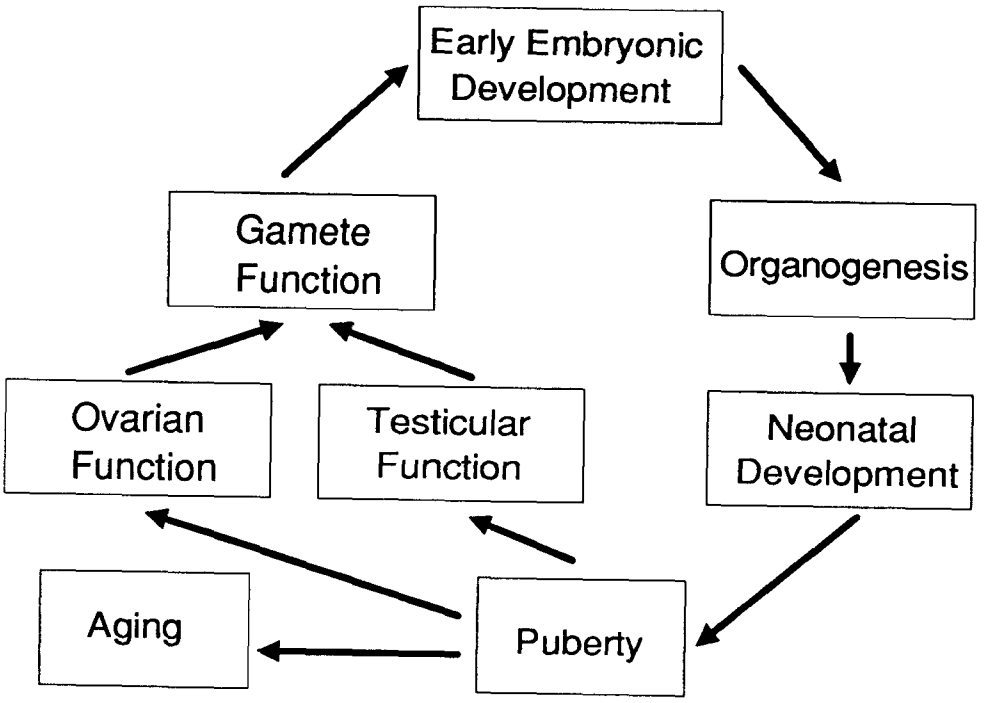

Fig. 2-7: Lifespan Reproductive Events

By focusing on the life cycle, the division has been able to divide its research activities into a continuum of smaller, well-defined processes. Relative sensitivities or progression of toxicities can then be followed through all the important stages of reproduction. Within the area of gonadal function, for example, concerns include endocrine and paracrine regulation of oogenesis and spermatogenesis; the production, release, transport, and maturation of the gametes; and the development of biomarkers of function that can be readily extrapolated to humans. If functional gametes reach the site of fertilization, the events of fertilization, cleavage and embryo transport, maternal hormonal milieu, and uterine receptivity associated with implantation become the focus of the DTD effort.

Once pregnancy has been initiated, a new period of special susceptibility is encoun-tered: embryogenesis. Here, DTD research efforts are concentrated on understanding the mechanisms of abnormal development; determining the significance of "minor" versus "major" malformations for extrapolation purposes; determining the relationships between pharmacokinetic and pharmacodynamic parameters; and assessing the significance of confounding factors such as maternal toxicity or potential reversibility of effects. To understand alterations in developmental processes, investigations of the physiology and biochemistry of postnatal animals is sometimes necessary because many of the organ systems (e.g., lungs) do not become operational until after birth. The neonate may also be at risk to toxicant exposure during the period when physiological functions are still developing into the adult capabilities.

The last major developmental hurdle is puberty, or the transition phase that culminates in full reproductive competence. This stage is used as a launching point to assess the overall effects of xenobiotics on reproductive processes, which might include a diminution in numbers or health of future generations. Finally, aging is the ultimate developmental process. Available data suggest that aging of the reproductive tract can be modified by toxicant exposures during critical preceding developmental periods. This lifestage may also pose significant new risks to individuals: their homeostatic processes may have diminished to the extent that they are less capable of withstanding previously innocuous xenobiotic exposures.

Human health effects caused by biotechnology agents can result from direct action of a microbe as well as through interactive mechanisms. With natural agents, direct effects such as infection, pathogenicity, toxicity, and oncogenic cell transformation are the basis for concern. In contrast, concerns with genetically engineered agents relate to more indirect mechanisms and center around the genetic instability that may result from dispersal of genes into other strains or species. Potential health consequences or inappropriate genetic interactions leading to the disruption of coordinated regulation of gene activity are therefore important considerations in deciding whether to allow their environmental release.

2.5.2 Division-Specific Research Needs

The importance of understanding and characterizing the human developmental and reproductive lifecycle

catalyzes a number of research needs in these areas in hazard identification, dose-response assessment, and chemical-specific information. These needs are separately categorized in the discussion below under reproductive and developmental toxicology and microbial pesticides.

2.5.2.1 Hazard Identification

Reproductive Toxicology. The relatively new discipline of reproductive toxicology presents four major challenges. First, evaluation of reproductive function in animals and humans requires a multifaceted approach, taking into consideration the marked physiological differences 1) between males and females, 2) among reproductively immature, mature, and senescent individuals of both sexes, and 3) among diverse but interdependent target organs (e.g., brain, pituitary, and gonads). Second, due to the relatively high background rates of infertility and spontaneous abortions in the general population, detecting and quantitating the potential of environmental agents to initiate adverse reproductive outcomes in humans is difficult. Factors responsible for the high background incidence are generally unknown. Third, outcomes resulting from acute or chronic exposures to reproductive toxicants are likely to differ with regard to their chronology, perseverance, and severity. These differences need to be defined and factored into the risk assessment process. Finally, reproductive toxicology must interface with the related disciplines of developmental toxicology (to meld fertility and adverse pregnancy outcome) and germ cell mutagenesis (to integrate the genetic and morphological integrity of the gametes to support normal development).

Reproductive toxicology has traditionally focused primarily on the male, with emphasis on direct testicular toxicity and the resultant decrease in sperm production. Current research issues revolve around defining endpoint relationships with respect to each other and to fertility, determining the significance of low-dose measures of effects, and evaluating the duration and reversibility of effects. However, an increased emphasis on the female is forecast as a result of recent reports of significant (about 25 percent) early, peri-implantation pregnancy loss in humans. In both sexes, the predictiveness of hormonal endpoints for evaluating loss of fertility must be determined, especially in the early stages of loss of reproductive competence.

Reproductive toxicity in animal models is presently evaluated through multigeneration or continuous breeding protocols: adult animals of both sexes are treated over periods of time that encompass at least one spermatogenic cycle in the male and two or more generations in the female. These protocols have serious shortcomings due to their reliance on fertility as their main endpoint. As pointed out in the proposed EPA reproductive risk assessment guidelines, fertility is an inadequate measurement on which to base assessment of reproductive dysfunction in rodents as many components of the reproductive axis can be compromised without affecting fertility. In addition, the present tests generally do not provide information on the affected sex, the likely target tissue of toxicant damage, or the mechanism of toxicity. Thus, a major need in reproductive toxicology is the improvement of testing protocols. Ideally, a flexible decision-tree approach should be developed for assembling an appropriate protocol in accordance with compound-specific information available at the time.

Also, a more comprehensive set of reproductive endpoints is needed in both the male and female to provide essential information about the affected sex and relative end-organ sensitivities. In the male, technological advances on semen analysis have been accomplished, including computer-assisted analysis of sperm motility; but these need to be rigorously evaluated in toxicologic and epidemiologic settings. In the female, methods to identify early pregnancy loss in animals are needed so that animal models can be developed for experimental toxicologic studies.

Developmental Toxicology. Current testing guidelines established by the principal regulatory offices are generally considered adequate for assessing the classical manifestation of developmental toxicity"embryonic death, gross structural malformations, and intrauterine growth retardation. However, for other major areas of concern, no acceptable detection methods are available. For example, there are currently no acceptable procedures for evaluating postnatal manifestations of developmental toxicity (including, but not limited to, effects on the central nervous system). This deficit exists despite the recognized inability of standard assays to evaluate alterations in organs and organ systems, such as the thyroid, adrenal, pulmonary, renal and immune, which remain quiescent until the postpartum period. Other key needs under hazard identification are techniques for developing structure-activity relationships, assessing the potential special susceptibilities of the lactating female and the pre-adolescent, and examining the relative strengths of *in vitro* test systems as primary screening versus mechanistic tools. Finally, the issue of transplacental carcinogenesis presents additional challenges to the risk assessment process.

Microbial Pesticides. In the past, microbial pesticide research efforts sought to improve the guidelines for safety assessments and registration standards of naturally occurring microorganisms. The present pesticide assessment guidelines for microbial pesticides appear to be adequate for most of the natural agents and simple genetic alterations. However, future efforts in genetic engineering will produce increasingly complex and heterogeneous lifeforms. An exotic array of agents possessing unique characteristics and novel modes of action is anticipated. The suitability of present tests for such agents must therefore be reassessed. Additionally, safety evaluation needs for nonpesticidal, genetically altered agents (e.g. bioremediation organisms) have yet to be clarified.

At present, the predominant uncertainties in assessing the risk associated with genetically altered microbial agents lie in hazard identification. Hazards associated with natural progenitor microbial agents and potential dangers associated with novel, genetically engineered strains have to be identified and characterized; and then tests must be developed to assess their potential to produce detrimental health effects. Such tests, when standardized and shown to be of general utility, can be used by the Agency for regulatory purposes, such as in subdivision M of the Pesticide Assessment Guidelines.

2.5.2.2 Dose-Response Assessment

Reproductive Toxicology. A clear need exists for research directed at the identification of toxic mechanisms, sites of action, cell/organ targets, and critical periods of vulnerability within the life cycle. This type of information is essential in establishing a biological basis for extrapolation of animal data to humans. Protocols using short-term exposures are particularly suitable for determining biologically based dose-responses using multiple endpoints, for evaluating reversibility, and for identifying effects during critical time periods (e.g., early pregnancy) or in specific populations (e.g., young versus old individuals).

Another major area of concern and research need is the identification of susceptible populations. For example, more information is needed about the potential of early lifetime exposures, especially to hormonally active agents at critical developmental stages, to affect the normal progression of puberty and sexual differentiation. Likewise, little is known about the relative sensitivity of aging individuals to reproductive effects, or about the potential of xenobiotics to accelerate the onset of reproductive senescence.

Developmental Toxicology. Clearly, the area of greatest need within the Agency for developmental toxicity data is the improved understanding and use of information generated in standardized test protocols. Short-term needs involve research to support the modification of these protocols to reflect greater understanding of the biological significance(s) of the various manifestations of developmental toxicity (e.g., skeletal variation, dilated kidneys, intrauterine growth retardation). This information would be viewed in terms of the risk equivalence and comparative expression of the four primary manifestations of developmental toxicity (death, structural modifications, altered growth, and functional alterations). Another major concern is determining the relationship(s), if any, between maternal toxicity and developmental toxicity. Can better indicators of each be developed and implemented? Are some forms of maternal toxicity casually involved in abnormal development? Answers to such questions will support greater confidence in the use of safety factors based on the most sensitive effect in the most sensitive species.

Longer-term needs entail the development of more comprehensive determinations of exposure and response variables in animal models, including incorporation of pharmacokinetic, pharmacodynamic, and quantitative dose-response models. Efforts must be directed at identifying and quantifying species-specific parameters that can be applied in mathematical modeling efforts, determining the relationship and time dependency between delivered dose and biological outcome, and characterizing the biochemical and molecular events that precede disruptions in morphological development. Ultimately, quantitative dose-response models linked with mechanistic information and physiologically based pharmacokinetic models will allow risk assessors to scale the developmental toxicity in accordance with known species-specific factors. Conceptual frameworks for this revolutionary approach to risk assessment need to be formalized, analyzed as to their underlying assumptions, and empirically validated.

Microbial Pesticides. Because inappropriate genetic interaction of genetically altered agents with other microbes that are either in the environment or endogenous in human cells, tissues, or organs could lead to detrimental health effects, the mechanism involved in such processes must be characterized. Interactive mechanisms could lead to the spread or acquisition of undesirable traits or enhance mechanisms that potentiate the occurrence of detrimental health effects. At present, few genetically engineered commercial products have reached the marketplace. Needs pertaining to dose-response assess-

ments for these products are presently being discussed within the Agency.

2.5.2.3 Chemical- or Agent-Specific Data

Reproductive and Developmental Toxicology. Defining research needs for chemical-specific information is difficult. Some program offices (e.g., Office of Drinking Water) may be able to lay out long-term regulatory agendas, and others (e.g., Office of Air Quality Planning and Standards) may be able to identify emerging research issues, but others require information on an ad hoc basis. Thus, OPP may be faced with a immediate need for data on a particular pesticide to make decisions about imminent hazard, or some national environmental disaster may dictate development of a critical database. DTD has traditionally been involved in both defined and unanticipated studies; and it has the staff and capabilities to meet such needs as they arise.

Methanol is currently a chemical of interest because its use as a clean fuel is expected to increase greatly. Evidence available to date indicates that methanol may represent a developmental and reproductive hazard. Therefore, a comprehensive evaluation of the effects of this compound is a current research need.

Microbial Pesticides. Because many of the genetically altered strains and natural progenitors used in microbial pesticides and other biotechnology products are frequently obscure agents, information such as specific strain identification and detection are required. Specific strain identification becomes critical for bacteria, since their major properties are frequently determined by dissociable extrachromosomal genetic elements (i.e., plasmids).

2.5.3 Research Plan

DTD's staff has traditionally been closely involved in risk assessment activities within the Agency. DTD scientists have served as members on committees drafting the risk assessment guidelines for developmental and reproductive toxicology, and have planned and participated in workshops and conferences to formulate recommendations for designing and interpreting studies that address major unresolved issues. In fact, they have often produced the key research papers that have formed the basis for many of these events and are routinely sought to participate in them.

Significant interactions"including the transfer of scientific and technical advice as well as research support"have occurred between DTD staff and the program offices. Not only do the risk assessment concerns of the program offices influence the research direction of the division, but the division's research outputs have had significant impact on both specific and generic elements of the risk assessment process, and thus on regulations governing various chemicals. In fact, the first decision to label a pesticide with information regarding potential adverse developmental effects, and the first decision to remove a herbicide from the U.S. market on the basis of developmental effects, were made on the basis of research performed by the staff.

Thus, through close interactions with other elements of ORD and the Agency, DTD maintains a high degree of programmatic relevance while advancing the state-of-the-science in developmental and reproductive toxicology. The research plan for each discipline has been crafted in light of the long-term needs of the Agency for improved detection, interpretation, and extrapolation of developmental and reproductive toxicities.

The specific goals in the area of microbial pesticides are to identify, characterize, and develop test methods for the assessment of health hazards associated with natural and genetically altered microbial pesticides and products of modern biotechnology. Because health effects research with microbial agents generally involves macromolecule and concerns center around genetic issues, a biomolecular approach is required.

2.5.3.1 Hazard Identification

Reproductive Toxicology. To address the need for improved reproductive tests, alternative reproductive toxicity test (ART) strategies are under development and validation. ART protocols may eventually replace or supplement the current multigeneration tests. DTD researchers will assess known reproductive toxicants using a protocol that begins with prepubertal animals and extends through sexual maturation, pregnancy, and the first generation. This protocol detects developmental effects during critical periods of sexual maturation, and at the same time evaluates the entire reproductive life cycle. It incorporates multiple endpoint measures, including cellular and endocrine measures, which will be evaluated for specificity and sensitivity. DTD's tiered approach incorporates the use of novel and sometimes more specialized endpoints when appropriate, particularly in follow-up, mechanistic studies. As research proceeds during the next

two to three years, the protocol will be refined and recommendations made for its incorporation into EPA risk assessment and/or testing guidelines.

To increase the number and usefulness of male and female reproductive endpoints, research efforts will improve methods for quantitative evaluation of gamete production and quality. Current work is focused on computer-assisted image analysis of rat sperm motility, and future work will extend this technology for morphometric analysis of sperm cells as well as testicular and ovarian histology. In the endocrine arena, a battery of hormone assays has been developed for use in rodent toxicology studies. Information provided by endocrine profiles will be compared with more invasive measures of reproductive dysfunction and their use as biomarkers of effects on the reproductive axis evaluated. A major advantage of both the endocrine and semen analyses is that similar measurements could be easily performed in humans with suspected exposures to environmental agents.

Developmental Toxicology. Within the next year, the DTD research program evaluating the relationships of maternal (adult) and developmental toxicity will have attained its major milestones. DTD researchers, however, will collaborate with scientists from NTD to continue the investigation of the role of maternal stress in altered embryonic development. This effort may define alternative methods of establishing the maximum tolerated dose levels used in standard developmental toxicity assays. DTD and NTD scientists will also work together to evaluate the effect of maternal separation (an experimental manipulation required for dermal or inhalation exposures during lactation) on postnatal growth, differentiation, and behavior. Results from this effort may ultimately feed into the developmental neurotoxicity testing guidelines that are currently being drafted.

DTD will also continue work in areas of interpretation of malformations versus "variations," focusing on the induction of supernumerary ribs. Research efforts will determine the mechanism behind this alteration, as well as how the endpoint is expressed across various laboratory species. Efforts to characterize SARs for substituted phenols and branched chain aliphatic acids will continue in support of the Office of Toxic Substances (OTS). Finally, DTD will continue to monitor the development and validation of *in vitro* teratology screening assays for potential applications in SAR as well as bioassay-directed fractionation projects.

Microbial Pesticides. Categorizing research under the hazard identification and dose-response assessment topics is more difficult for microbial pesticides than for chemical agents. Therefore, these types of efforts are addressed together here.

Some of the proteins contained in spores of the registered pesticidal agent, *Bacillus thuringiensis* subsp. *israelensis*, are toxic to mammals. DTD is investigating the precise nature of the physiological effects, the mode of action, the molecular genetics, and the potential for mutagenic inactivation of the toxin gene. Aside from providing data for health effects assessment of the toxin, the assignment of the mammalian toxic activity to a specific gene product distinct from the insecticidal components will prevent mistaken use of the mammalian toxin gene in genetic engineering of other bacteria and plants.

Inappropriate interaction between the altered agent and other microbes inhabiting the environment or human cells and tissues may lead to detrimental human health effects. An ongoing investigation is characterizing mechanisms involved in the movement of a marker mammalian toxin gene between bacterial chromosome and other genetic elements, as well as other bacteria strains and species. The results of this work will provide a better understanding of how an unstable engineered gene could be transferred (or detrimental genes acquired) by genetically altered agents to produce agents which then could induce adverse health effects. Inappropriate genetic interactions could also occur at the molecular level, resulting in the activation of latent endogenous agents. Current research is investigating the potential for activation of human cytomegalovirus infections by genetically engineered baculoviruses.

Finally, DTD is developing more appropriate and sensitive tests for assessing the health effects of microbial agents used as pesticides. The division is attempting to incorporate state-of-the-art technology into preregistration tests recommended by the Agency for candidate viral pesticides; this effort should increase their reproducibility, sensitivity, and cost effectiveness. These new tests will be standardized, and the general efficacy over a range of human and other nontarget species cell cultures determined. Special emphasis will be placed on developing tests to assess the likelihood and potential effect of foreign genes moving into human cells from engineered organisms.

2.5.3.2 Dose-Response Assessment

Reproductive Toxicology. In response to the need for short-term reproductive assessments, current efforts are directed at evaluating the pathogenesis of known reproductive toxicants. A variety of cellular and endocrinologic endpoints are being applied and compared for relative sensitivity and predictive value. Researchers are periodically evaluating endpoints over a short-term period to allow identification of the primary site of lesion (hypothalamus, pituitary, gonad) and localization of cellular targets in the gonad. This approach is being used to evaluate susceptible populations (e.g., effects in young versus old animals) and to compare acute and subchronic effects. The long-range goal of these studies is to make recommendations regarding choice of endpoints in reproductive toxicology and extrapolation from acute to (sub)chronic effects in specific categories of reproductive toxicants.

The DTD program is also developing and applying *in vitro* tests using reproductive cells and tissues to conduct mechanistic studies and to provide data for interspecies extrapolation. For example, *in vitro* production of steroids by Leydig cells from the testes or granulosa cells from the ovary are being used to dissect effects on the steroidogenic pathways in animal models. Likewise, secretion of proteins from epididymal epithelial cells is being examined in an attempt to find improved biomarkers of epididymal function that may one day be applied to analyze human semen. Other cellular approaches under evaluation include *in vitro* tests to detect local changes in neuroendocrine function (such as hypothalamic and pituitary perfusion) and to assess gamete function (*in vitro* fertilization, sperm microinjection). Hormone receptor assays and biochemical analysis of placental tissues during implantation are also under development.

In response to the need for enhanced efforts in female reproductive toxicology, research efforts under the Research to Improve Health Risk Assessment (RIHRA) program will examine the homology between animal models and humans regarding ovarian and uterine function. Short-term tests will continue to be used to assess effects of chemicals on the luteinizing hormone (LH) surge that triggers ovulation, on the fertilization process, and on the initiation and maintenance of pregnancy. Again, multiple endpoints will be compared for their relative utility, and recommendations will be made regarding identification of hazards to early pregnancy in animals and hence to humans.

Developmental Toxicology. The long-range goal in this area is to develop procedures for pharmacokinetic and biologically based dose-response assessments of developmental toxicity. Pharmacokinetic studies will initially focus on theoretical aspects of placental transport and embryonic deposition of a series of phenols and aliphatic acids used in previous SAR studies in the lab. These data will be used to define the relationships among administered dose, delivered dose, and developmental outcome. Researchers will compare results from both *in vivo* and *in vitro* systems and will evaluate physiologically based pharmacokinetic models as a tool in the risk assessment process. Other pharmacokinetic studies will evaluate the inter-litter (maternal), intra-litter (uterine position), and embryo (genomic) sources of variation in developmental response. In addition, efforts will continue in evaluating the role of the vehicle in bioavailability and in the relative importance of peak versus total exposure estimates for the developmental toxicities induced by disinfectant byproducts. Through co-cultures of mammalian embryos and heterologous metabolic activation systems, the role of pharmacokinetic versus pharmacodynamic factors in species sensitivities will be approached. Finally, through techniques of biochemistry, cell biology, and flow cytometry, early events leading to stage and species-specific alterations in embryonic development will be probed to elucidate mechanisms of abnormal development and to evaluate the existence of thresholds. DTD will assemble the information compiled in the various components of this research to produce biologically relevant quantitative dose-response models.

Other work involves better use of available information on human developmental toxicants. Examining the animal literature on these agents highlights the tremendous gaps in knowledge of these toxicants' dose-responses in standard animal models. Many of these agents have defined pharmacokinetic and pharmacodynamic profiles that could be readily compared across species. So-called retrospective risk assessment can be used to evaluate how a well-developed animal database predicts known human risk. Lessons learned from this endeavor will feed back into improved "prospective" risk assessments for developmental toxicity.

2.5.3.3 Chemical- or Agent-Specific Data

Reproductive and Developmental Toxicology. A small proportion of DTD's research strategy is directed at chemical-specific information for developmental toxicology. Of that, the largest component has been catalyzed by the Office of Drinking Water's (ODW's) need to evaluate health hazards imposed by drinking water disinfectants

and their byproducts. Given the types of compounds involved, this information should ultimately be useful in SAR evaluations. The induction of cardiovascular malformations by several related disinfectant by-products will form the basis for a broader-scale analysis of the dose-response characteristics, the nature of the biochemical lesion, and the potential interactions of simultaneous exposure to multiple disinfectant by-prod-ucts. SAR studies involving MX-type chemicals are also a possibility.

OPP has sporadic but continuing needs for specific research efforts on particular pesticides. DTD's involvement is determined by the criticality of the need in terms of time, the ability to utilize state-of-the-science technology to answer a perplexing problem, and/or the need to arbitrate conflicting data. OTS has interests in SAR evaluations on specific chemical classes, such as the aliphatic acids for which DTD will be constructing the necessary databases over the next several years. DTD will also continue to coordinate efforts to develop Developmental Toxicity Activity Profiles analogous to those developed for genetic-toxicity bioassays.

In response to the Agency's need for information regarding the reproductive effects of methanol, short-term dose-response and route-to-route studies have been initiated using endocrine assessments and testicular/sperm evaluation in male rats. Effects are related to blood methanol levels as well as applied dose. The acute effect of methanol on the LH surge in females is also under investigation; and, pending results, effects on fertilization and preimplantation development will also be explored. In addition, researchers will evaluate the adverse effects on prenatal development, including alterations in the function of the nervous system in postnatal animals and on development of a database on a second rodent test species.

Microbial Pesticides. A major problem for the Agency in regulating microbial pesticides is definitive identification and differentiation of bacterial strains. This problem has been exacerbated by modern biotechnological methods in which genes are readily moved between strains by recombinant DNA methods and mobile genetic elements. One answer to the problem is to obtain a genetic map of the bacterial chromosome. In conjunction with studies on the movement of the bacterial mammalian toxin gene described above, investigations are underway to identify sites on the bacterial chromosome that can be used to construct a genomic map. Fine genetic maps showing unique positions of genes will permit differentiation of closely related strains for regulatory purposes by the Agency.

2.5.4 Emerging Issues

2.5.4.1 Improvements in Risk Assessment

Over the next several years, the Division will continue to focus on detecting, interpreting, and extrapolating test results to the human situation. Advancements in risk assessment will follow the interactive process in which improvements in detection lead to questions of interpretation"which, when answered, clarify the extrapolation process. Likewise, improved recognition of the weak points in risk assessment yield research questions addressed by the detection and interpretation components of the mission. Research in developmental toxicology will become increasingly focused on the extrapolation process, while that in reproductive toxicology will be moving more into the interpretation and then extrapolation areas.

To accomplish these goals, greater emphasis must be placed on the development of more integrative approaches by the various research teams. Greater coordination of the study of metabolism, distribution, morphology, biochemistry, and physiology as related to toxicological mechanisms is required. Additional resources will have to be placed in both the pharmacokinetic and pharmacodynamic efforts. Rapid advances now occurring in cell biology and morphology will have to be incorporated into all research endeavors, a feat that will require additional scientific training and staffing in areas such as biochemical toxicology, molecular biology, and experimental morphology. All research programs will need a greater understanding of the concentrations of the proximate toxicant in their target tissue. More involved interactions between scientists of complementary expertise will be required to provide integrated assessments of all aspects of developmental and reproductive risk. Methanol may be a prototype chemical with which to utilize this combined expertise.

2.5.4.2 The Female as a Susceptible Population

The female as a susceptible population is an emerging research issue that needs the support of an ovarian, placental, or lactational physiologist. In the area of male reproductive effects, DTD has no expertise in the area of risk of heritable genetic damage to germ cells. This is an important aspect of evaluating the total risk to reproductive and developmental toxicants, and all would benefit by closer collaboration with scientists in GTD who are addressing this issue. In developmental toxicology, an emerging need for research in developmental neurotoxicity is clearly indicated. Collaborations with scientists

in DTD and NTD is needed to situate HERL in a position to address this issue.

2.5.4.3 Nontraditional Exposure Regimens

Another emerging issue relates to nontraditional exposure regimens. "Traditional" in this context refers to single daily oral exposure to individual agents. The nontraditional exposures, in contrast, relate to considerations of dose-rate effects, dermal and inhalation exposures, multiagent (i.e., complex) exposures, and the use of pharmacokinetic information to design more appropriate exposure scenarios or to extrapolate results between various routes of administration. Factors relating to study design (e.g., the impact of maternal isolation on neonatal development during dermal or inhalation exposures) are included in this concept.

2.5.4.4 Age and Toxicity

As the population ages, interest is increasing regarding the influence of toxicants on the aging process and the influence of aging on toxicity. Within the existing structure, DTD has the base capability of establishing an aging animal colony for use by HERL staff in such disciplines as pharmacokinetics, neurotoxicity, cardiovascular, immunology, and pulmonary function. The effects of aging in terms of reproductive senescence will continue to be an integral component of DTD research.

2.5.4.5 Biotechnology

In the area of biotechnology, the division maintains expertise in the areas of viral and bacterial pesticides but lacks a specialist in fungal biology. Representatives from this phyla are likely to be developed as biological pesticides in the coming years, and DTD will be able to advise OPP on health issues related to fungi, as is already done for other types of biological agents.

2.5.5 Summary

2.5.5.1 Hazard Identification

In reproductive toxicology, DTD will improve the techniques used to detect and characterize impairments in fertility and fecundity. Better detection procedures are available in developmental toxicology; thus, scientists in this area will focus on improving the extrapolation of toxicologic data. To accomplish these objectives, the division will shift resources away from describing toxicological manifestations toward examining the proximate events in the induction of abnormal morphological and functional development as well as reproductive dysfunction. Blends of *in vivo* and *in vitro* methods and biochemical and morphological approaches will be used to develop comprehensive pictures of the full spectrum of developmental and reproductive alterations.

2.5.5.2 Dose-Response Assessment

In close conjunction with efforts to biologically characterize toxicity, scientists will use radioisotopic labelling, autoradiographic techniques, and analytical chemistry to analyze target tissue disposition of xenobiotics. Where possible, the biological and pharmacokinetic efforts will concentrate their joint activities within chemical classes (e.g., phenols, aliphatic acids) so that structure-dosimetry-activity relationships can also be derived.

DTD will continue to assess the postnatal functional consequences of prenatal insults, though at a slightly reduced level of effort. Researchers will complete the analysis of effects of maternal deprivation on postnatal growth and maturation, and these data will be used to establish testing guidelines for dermal and inhalation exposures over the perinatal period.

In reproductive toxicity, research effort to identify more sensitive and comprehensive endpoints for assessing reproductive dysfunction will culminate in improvements to the Agency's testing and risk assessment guidelines. Emphasis will be placed on developing biomarkers of reproductive function for both the male and the female.

To support the program offices, DTD will assess a wide variety of drinking water disinfectants, the reproductive and developmental effects of existing and alternative fuels, and the appropriateness of the additivity assumption for mixtures.

To improve risk assessments, researchers will use data gathered in various DTD efforts as input for empirical and biologically based quantitative dose-response models. Various complex issues associated with the use and development of these models will be addressed.

DTD is also developing and validating methods to screen and quantify the potential adverse health effects associated with microbial pest control agents (MPCAs). Researchers will examine the mechanism of mammalian toxicity that results from exposure to components of a bacterial pesticide. This information will then be used to

develop specific assays for their identification. These same methods can be applied to genetically engineered organisms. DTD will assess the potential for interaction at the genetic level and for transfer of engineered genes of viral biotechnology and pesticidal agents that enter human and other mammalian cells. Researchers will determine whether genes present in human or other mammalian cells can be activated by biotechnological agents and, if so, to what level. To assess the importance of gene transfer as a route by which genetically engineered agents can cause health effects, DTD will also investigate the movement of genes for mammalian toxins between genetic components of a bacterial pesticidal agent.

2.6 HUMAN STUDIES DIVISION

2.6.1 Divisional Program

Investigators in the Human Studies Division (HSD) study people's responses to exposure to pollutants and mixtures of pollutants commonly found in the environment. Human research conducted in HSD has immediate, direct, and significant application to the regulatory and rule-making activities of the Agency.

HSD's research program encompasses all aspects of health research"exposure, dose, and effects. Determining exposure is particularly important in HSD's epidemiologic and field studies, which are generally conducted in natural, real-world settings. (In the division's clinical and *in vitro* studies, on the other hand, exposure levels are known and carefully controlled.) In an effort to interpret mechanisms of action and to extrapolate data from one set of conditions to another, HSD is also exploring the relationship between exposure concentrations and the amount of pollutants or their metabolite(s) that reach target tissues and cells in humans (i.e., the dose). With these data, HSD can work to characterize the risk associated with exposure to a given pollutant by establishing relationships among exposure, target dose, and adverse health consequences. Improved risk characterization results in better risk assessments and in more accurate monitoring of the effectiveness of risk reduction strategies.

In HSD clinical studies, human volunteers are exposed to common environmental pollutants in laboratory "chambers" under carefully controlled and monitored conditions. The pollutant levels are comparable to or less than ambient exposures in many urban areas and are expected to cause only reversible, transient effects such as symptoms, decrements in pulmonary function, and biochemical changes. HSD scientists note the acute effects experienced by the volunteers as a result of the pollutant exposures and assess whether longer-term or higher-level exposures to the same pollutants could cause disease. They also conduct dosimetric studies on the absorption, delivery to target sites, and elimination of environmental materials.

Sample tissues, cells, and lung fluids are collected for *in vitro* studies (e.g., by nasal or bronchial washing or by epithelial scraping) during clinical studies. *In vitro* studies, which are conducted with tissues, cells, and cell lines obtained from volunteers, enable HSD investigators to obtain very specific data on cell function; these data contribute to our understanding of the immunotoxic and cytotoxic mechanisms of effect that pollutants have on human health. Such studies are especially important for gathering exposure data on pollutants to which humans cannot be exposed experimentally. Both clinical and *in vitro* exposure data are used to identify biomarkers of exposure, which can then be tested in field studies.

In epidemiologic and related studies, HSD investigators look for patterns of biological effects (pre-clinical and clinical), disease, and mortality in groups of people who have been exposed, over both long and short terms, to pollutants in their living or working environment. Such studies are a vital means of collecting effects data on pollutants to which humans cannot be exposed experimentally. Epidemiologic work is also used to monitor the effectiveness of risk reduction strategies, i.e., to determine whether reduced pollutant levels have benefited human health. Field studies can address these same issues, as well as provide a means to quantify the incidence of disease and death in humans. Combining clinical, *in vitro*, and epidemiologic data allows HSD researchers to identify potential human health effects associated with exposure to pollutants and verify their occurrence in real-world settings. Together, these three types of studies give HSD the opportunity to study human responses to a wide variety of exposures"ranging from uncontrolled natural exposures to highly controlled laboratory exposures"and to study a wide range of effects at different levels of biological organization (whole body, systemic, cellular, and biochemical). With such an integrated approach, investigators can better understand the dose of pollutants delivered to the individual and to the target site under a variety of exposure conditions, as well as the effects of the exposure and the mechanism of the effects in man. In addition, human research offers the opportunity to validate postulates developed in animal models and to evaluate many risk assessments that are based on theoretical and experimental efforts.

HSD research has helped serve as the basis for several important regulatory decisions by the Agency, including the current NAAQS for ozone (based on both clinical and epidemiologic data); the 10-micron standard for particulate matter (epidemiologic data) and the recent recommendation by the Science Advisory Board that a NAAQS be set for acid aerosols (based on epidemiologic data). HSD epidemiologic data have also demonstrated that improved ambient air quality resulting from clean air regulations has resulted in an improvement in human health.

2.6.2 Division-Specific Research Needs

2.6.2.1 Hazard Identification

One issue of major importance to risk assessment is how much of the pollutants to which we are exposed reaches target organs. Improved and novel dosimetry methods are needed to answer this question. Data from dosimetry studies is being used to evaluate the potential human health effects of exposure to inhaled environmental pollutants and to develop mathematical models of how the human respiratory tract handles such inhaled pollutants. This research needs to be carefully integrated with the animal dosimetry program conducted in the ETD.

Environmental research requires innovative approaches and a wide variety of methods to test for different endpoints and to understand the effects associated with low levels of exposure to pollutant(s) on a variety of biological and health endpoints. Improved methods are also needed to measure a wider range of biological, preclinical, and clinical endpoints more accurately. New tests that are more sensitive to subtle changes and that focus on specific areas known to be susceptible to injury need to be developed and validated. For example, small airways, which constitute a large portion of the surface area in the lungs, are thought to be particularly vulnerable. Dosimetry and modeling studies indicate that small airways are the site of greatest deposition for many airborne pollutants. Epidemiologic and animal data have shown that pulmonary responses and morphological changes occur in the small airways of the lung in response to pollutant exposures; however, detecting such effects in humans in clinical settings has proved particularly difficult. More sensitive methods are clearly needed for detecting effects on the small airways, as well as on other tissues and systems.

Research is also needed to increase the sensitivity and specificity of the exposure and disease endpoints used in epidemiologic research. In addition, HSD epidemiologists need:

- Methods for determining the best available exposed populations and study opportunities
- Modeling approaches for studying the feasibility and optimal design of proposed studies
- Information on the extent to which modern computerization of health records (such as registries and hospital admissions) can facilitate cost-effective monitoring of health effects associated with pollution
- Methods for subclassifying diseases into components that are related and unrelated to specific environmental agents
- Methods (measurements) that effectively estimate population or person exposures in epidemiologic studies

2.6.2.2 Dose-Response Assessment

Much HSD research has focused on establishing dose-response relationships for a variety of inhaled pollutants, particularly NAAQS pollutants. This research has identified a range of acute effects found in different human populations or subpopulations following short-term exposure to the pollutants"including bronchoconstriction in asthmatics after exposure to sulfur dioxide, loss of pulmonary function in individuals who exercise in ozone, or decreased vigilance due to carbon monoxide exposure. Such acute responses are considered adverse because they could interfere with the normal activities of daily living or significantly impair human performance capabilities.

A key question that needs to be addressed through mechanistic studies is the relationship between exposure and delivered dose to the target organ. Historically, HSD research has emphasized uptake of gases and particles by the lung. Physiologically based pharmacokinetic studies are needed to investigate the absorption and fate of pollutants in the lungs and other systems, such as the skin and the gastrointestinal tract.

Research is also needed to understand the mechanisms of response to pollutants. For example, when a responsive individual is repeatedly exposed to ozone on consecutive days, an initial increase in pulmonary function

response (on day two) is followed by a progressive and remarkable attenuation in response: By day four or five, no measurable functional response to the exposure can be measured. Does this attenuation represent a positive adaptation, or does it mean that the epithelial cells have been damaged and are no longer able to respond to the challenge of inhaled pollutants? Will repeated exposures to ozone also attenuate the immune and inflammatory responses? The answers to these questions have major implications for the regulation of air pollutants.

Some individuals or populations (e.g., the elderly, children, immunocompromised individuals, and patients with chronic obstructive lung disease or ischemic heart disease) are more responsive to a given pollutant than are others. This increased responsiveness to pollutant stimuli can be caused by factors such as preexisting disease, other current exposures, individual sensitivity, or perhaps genetic predisposition. These populations or individuals may be particularly sensitive to pollutants such as ozone, sulfur dioxide, carbon monoxide, and volatile organic hydrocarbons. To improve risk assessments and thus regulatory decision-making, research is needed to identify sensitive populations, determine the reasons for their sensitivity, and characterize their responses to various levels of exposure. The Clean Air Act of 1970, as amended (1977), for example, mandated that protection from airborne pollution be extended not only to normal, but also to the most sensitive, individuals in the population. In addition, work with sensitive individuals may shed light on the mechanisms causing air pollution effects.

One particularly sensitive population to certain air pollutants is asthmatics. Asthma hospital admissions are increasing in the United States, and little is known regarding the causes"though some theories point to air pollution. Asthma, which is a disease characterized by hyper-reactive airways, can be life threatening. Epidemiologic studies have shown that asthmatic subjects are at added risk during periods of increased air pollution, though controlled laboratory studies indicate that asthmatics are particularly responsive to some"but not all"pollutants. Important questions remain regarding the response of asthmatic individuals to air pollutants and the role of air pollutants in the development or exacerbation of asthma.

Due to the limitations on conducting experiments with human subjects, most data used to assess risk to human health from exposure to various pollutants are gathered from studies on laboratory animals. Currently, however, much uncertainty surrounds the extrapolation of data from animals to humans and from the high doses commonly used in the laboratory to the low doses to which humans are usually exposed. To determine which animal models can most accurately predict human health effects, scientists must compare the pollutants' mechanisms of action in humans with those in various laboratory animals. Such research contributes substantially to improvements in health risk assessment.

Epidemiologic and animal research has demonstrated changes in host defenses associated with exposure to environmental pollutants: For certain individuals, exposure appears to increase susceptibility to respiratory infections. Additional epidemiologic research evaluating the associations between pollution episodes and illness (perhaps using hospital records) is needed to assess these risks. In addition, clinical research across a broad range of host defense mechanisms, including deposition and clearance of inhaled materials, is needed for this task.

Understanding the relationship between acute responses and the development of chronic disease is also of critical importance in risk assessment. For example, the immunotoxicological and inflammatory response that has been observed in human lungs following exposure to environmental pollutants is a potential stage in the development of chronic changes in the lung. Research is needed to explore the connection between the acute responses observed in clinical studies and later chronic effects.

2.6.2.3 Chemical-Specific Data

Toxics and Superfund. Many chemicals (e.g., PCBs, TCDD, phosphene, lead) known to be toxic or found on Superfund sites raise public health concerns. Studying the health effects associated with exposure to each one of this large number of chemicals would be impossible. Therefore, research efforts must be directed to understanding principles of action so that risk associated with exposure to various compounds or toxic chemicals can be extrapolated from available data on other chemicals or compounds. Investigations with human tissues will involve primarily immune host defense studies *in vitro*.

Acid Aerosols. Acid aerosols are another group of chemicals for which additional health effects data are needed. Cumulative evidence from animal, clinical, and epidemiologic studies clearly suggests that health effects can be associated with exposure to acid particles. Based on this evidence, the Science Advisory Board's Clean Air Science Advisory Committee recommended to the Administrator in October 1988 that acid particles be considered for listing under Section 108 of the Clean Air Act. Within 12 months of a listing decision, the Agency would

have to issue air quality criteria and propose standards. Because the database for acid aerosols is incomplete, however, research is needed to determine whether the listing is justified and, if so, to provide a basis for developing criteria.

UV-B. Environmental pollution is thought to be depleting the ozone layer in the earth's stratosphere, thus increasing human exposure to ultraviolet (UV) radiation from sunlight. Available animal data suggest that such exposure may have both local and systemic immunosuppressive effects. Suppression of the immune system increases the risk of disease, especially infectious disease, and may also contribute to higher rates of cancer. Research is needed to investigate whether the correlation between immunosuppressive effects that has been shown in laboratory animal models is also valid for human health effects.

Ozone. Data from both humans and animals strongly suggest that repeated and long-term exposure to ozone can be associated with the development of chronic illness. This connection poses a risk for individuals living in areas where ozone level requirements have not been attained (an estimated 100 million people in the continental U.S. alone). Epidemiologic and clinical studies are needed to assess this risk for people who live in both urban and rural areas and who are exposed repeatedly and chronically to ozone and associated pollutants.

Drinking Water. Chlorination of drinking water has reduced the waterborne transmission of infectious disease. Recently, however, a variety of organic contaminants, both volatile and nonvolatile, have been identified as resulting from the reaction of the chlorine with naturally occurring humic substances. With *in vitro* tests, researchers in other divisions of HERL are identifying the various chlorination byproducts, assessing the compounds' toxicity to animals, and examining the contaminants' mutagenicity. Epidemiologic studies are needed to determine the cancer and cardiovascular risks associated with water chlorination.

A number of epidemiologic studies have reported an association between certain cancers and chlorinated water, and analytical epidemiologic studies have shown a moderate increase in risk of bladder and colon cancer in populations with a relatively long duration of use of chlorinated drinking water. Establishing any causal associations between water chlorination and these cancers, however, must await the completion of additional epidemiologic studies as well as other research.

Indoor Air. Relatively little is known about human physiological responses to indoor air pollution, which accounts for much lost work and productivity (the "sick building" syndrome). Symptoms associated with the syndrome suggest irritation of the upper airways, but more research is needed to pinpoint mechanisms of response to the pervasive and ubiquitous compounds found in indoor air.

Exposure to radon is thought to be responsible for most lung cancers occurring among nonsmokers, and it probably also contributes to smoking-related lung cancers. In some areas of the United States (e.g., Maine), drinking water contains very high levels of naturally occurring radon. Volatilization of radon during water use (e.g., showering) can be the major contributor to radon in indoor air. Research is needed to determine whether this source of radon results in significant exposure.

2.6.2.4 Biological Markers

One important area for additional research is the development of biomarkers of exposure and response. Biomarkers of exposure are any biologic measurements (e.g., in tumors or blood specimens) that can be used to detect responses and quantify exposure to pollutants, either systemically or in critical target tissues. Biomarkers of effect are biologic responses that occur in conjunction with or prior to the development of adverse health effects. They are important tools in detecting and predicting effects. Clinical, *in vitro*, and epidemiologic research all contribute to the development of these biomarkers.

2.6.2.5 Pollutant Mixtures

Most pollutants are found in the environment in mixtures of multiple pollutants rather than in pure form. Both epidemiologic and clinical studies are needed to evaluate how exposure to pollutant mixtures is different from exposure to single chemicals. For example, HSD investigators need to examine whether pre-exposure to one pollutant potentiates (or blunts) the response to exposure to a second pollutant and whether combinations of pollutants act synergistically.

2.6.3 Research Plan

HSD's research program is divided between two of the division's branches: the Clinical Research Branch and the Epidemiology Branch. Considerable effort, however, has been devoted to integrating these research activities

across branches. For example, new tests developed and tested in the clinical facility may be applied in epidemiologic studies conducted primarily in the field. Similarly, clinical research lends itself to identifying responsive or sensitive populations, who HSD epidemiologists can then use as target populations, and vice-versa.

2.6.3.1 Hazard Identification

Dosimetry. Work in this area will be directed to better understanding the relationship between exposure concentrations of air pollutants and the dose of those pollutants or their metabolite(s) that reaches target organs. The total deposition of gaseous pollutants at various points in the human respiratory tract will be measured experimentally. In addition, HSD investigators will evaluate the locations in the respiratory tract where the compounds are deposited and the rates at which they are cleared.

The size of an inhaled particle influences its trajectory, which in turn influences where it is deposited. Many inhaled pollutants grow in size due to moisture acquisition as they travel through the respiratory tract. Therefore, techniques are being developed to measure particle growth in the human respiratory tract to facilitate understanding of patterns of particle deposition and to develop models of the transport process.

Development of Sensitive Pulmonary Function Tests. Tests of pulmonary function are commonly used to measure responses to inhaled air pollutants in humans. Tests believed to be sensitive to small airway dysfunction are being adapted and evaluated for use in human environmental research. Such tools should enable measurement of subtle effects much earlier than classical pulmonary function tests presently allow"an improvement that could make clinical studies more sensitive in detecting these effects.

These tests, as well as other laboratory test methods, are being adapted for use in the field. For example, researchers are examining the validity of using heart rate as an indicator of ventilation rate in subjects who exercise outdoors. Pollutant dose rate (calculated using the ventilation estimates from the heart rate and the measured ambient pollutant concentration) can be correlated with measured changes in pulmonary function. HSD researchers use portable monitors to measure ambient pollutant levels and calculate pollutant dose rate"measurements that can then be correlated to changes in pulmonary function. This approach could provide a relatively simple way to field validate some of the effects data that are obtained from clinical studies.

With nasal and bronchoalveolar lavage techniques, HSD investigators can sample tissues, cells, and fluids from either the upper or lower airways in humans. The samples are gathered either from naive (unexposed) subjects for use in *in vitro* studies or from volunteers previously exposed to environmental pollutants in order to examine the effects of inhaled pollutants on respiratory cell biology and immunology. Though the bronchoalveolar lavage technique must be conducted by medically qualified personnel and demands the highest operational standards for application in this context, the resulting data are crucial to an understanding of the mechanisms of response and health effects at the cellular and molecular levels. The nasal lavage procedure, on the other hand, is relatively simple and noninvasive. Because of its simplicity, the nasal lavage procedure may also have useful applications in field studies. The application of novel molecular techniques to these types of study will improve our ability to detect changes in cells exposed to pollutants and provide new endpoints that could be used as biomarkers of exposure.

Other methods are currently being developed to analyze whether and how pollutants affect human resistance to infection. In one effort, HSD investigators are developing techniques to grow human epithelial cells and cell lines in culture. The objective of these studies is to determine the susceptibility of these cells to viral infections, and their contribution (e.g., mediators and cytokines) to the inflammatory response following exposure to pollutants. Epithelial cells are the first target for exposure in the upper respiratory tract, and their ability to remain functional and anatomically intact is important in maintaining normal lung homeostasis.

According to available animal data, some behavioral responses can result from exposure to a number of pollutants. HSD researchers are developing and adapting new tests of neurobehavioral function and validating them for use with volunteers. These tests, which are more sensitive and specific than those currently available, will allow behavioral responses to be measured directly in humans. For example, research will be directed to determining whether changes in the visual evoked potential (the measure of electrical brain activity in response to a visual stimulus) can be used as a predictor of visual disfunction. If evoked potential is a biomarker of effect, it will be an important tool for future studies of the neurobehavioral effects of individual pollutants.

In addition, more objective measures for behavioral responses are needed to study areas of potential effects, such as the sick building syndrome, that are now identified largely by anecdotal data. A computerized battery of neurobehavioral tests is being validated and tested that should permit analysis of larger groups of people. This computerized test battery may have important applications in field studies, where larger numbers of individuals from exposed populations need to be tested.

Most of the tests described above have potential application in both clinical exposure studies and in epidemiologic (field) studies.

Drinking Water. HSD epidemiologists will continue to support local public health departments and other federal agencies in determining whether specific outbreaks of infectious diseases resulted from faulty filtration or disinfection of drinking water. These outbreaks provide the opportunity to refine the epidemiologic methods used for answering this question.

2.6.3.2 Dose-Response Assessment

Dosimetry. Ongoing studies on the dosimetry of gases and particles in humans will continue in order to increase the accuracy of animal-to-human extrapolations of data. These studies examine the dose to human target tissues from exposure to air pollutants as well as the mechanisms of delivery of these materials, including location of distribution and quantity deposited. By closely coordinating HSD's dosimetry efforts with those in ETD, HERL researchers can compare human and animal data to determine which animal models most closely mimic the human deposition pattern for particular pollutants.

Mechanisms of Response. A major focus of human research is the mechanisms of response to air pollution. This research is coordinated with animal studies developing parallel models and projects. A concerted effort is also being made to integrate epidemiology and clinical research"particularly in the development of biomarkers and in understanding associations between acute responses observed under conditions of acute exposure (such as in experimental laboratory [clinical] or field studies) and the chronic changes that might be produced following long-term daily exposures (such as in many urban or rural areas in the United States).

Both for scientific and regulatory purposes, the Agency needs to define the mechanisms of response to environmental pollutants; and HSD is pursuing several important lines of investigation in this direction. One effect of pollutant exposure can be the development of an acute inflammatory response evidenced by an influx of neutrophils and inflammatory mediators into the lungs. Understanding the significance of this response may allow researchers to answer the question: Do repeated bouts of inflammation lead to chronic damage in the lungs, as might be expected because of the presence of materials associated with both destructive and fibrotic processes; or is the lung capable of resolving these acute incidents as they occur?

Another important mechanistic question possibly associated with the inflammatory response is whether repeated intermittent and/or daily exposures over a period of many years leads to or contributes to chronic problems in individuals or groups of individuals. A multiyear and multidisciplinary study to investigate how the acute responses measured in clinical and field studies relate to the development of chronic respiratory disease and decrease in pulmonary function is being evaluated. This study would require 2-3 years of pilot research and then sustained funding for 10-12 years. Defining an association between the acute response to pollutants experienced by some individuals (e.g., to ozone) and the ultimate development of chronic pulmonary disease would have a profound effect on the scientific and regulatory community.

Another important issue is the mechanism by which the physiological response of many individuals to repeated sequential exposures is attenuated. An understanding of this phenomenon requires research efforts on the pulmonary function (airways), inflammatory, and immunologic responses in humans.

Sensitive Individuals. HSD has studied a large number of subjects at 0.18 ppm ozone to better define the distribution of responsiveness to this pollutant in a normal population. Some of these subjects, when re-exposed one or two times, were found to have reproducible responses"a finding that indicates that some individuals are consistently more responsive than others. Although this effort has provided useful data for risk assessment, much additional work is required to identify the determinants of this variability in responsiveness.

In addition, numerous studies have provided an extensive database concerning the response of asthmatics to sulfur dioxide exposure. In most of these studies, young, generally unmedicated, mild asthmatics have undergone short-term sulfur dioxide exposure at increased ventilatory

rates (exercise or eucapnic hyperventilation) with a variety of coexisting variables. Although asthmatics appear not to be hyper-responsive to ozone, as measured by symptoms or pulmonary function, their responses to other provocative agents (e.g., antigen) and bronchoconstrictors may be intensified following ozone exposure; and these responses should be investigated.

In addition, because epidemiologic evidence suggests that air pollution may increase the incidence of asthmatic attacks, longer-term exposures (i.e., extended exposures on several consecutive days) will be analyzed to determine if such exposures contribute to the development or exacerbation of asthmatic attacks. Such studies are also needed to evaluate whether the incidence of asthma increases with elevated ambient pollutant concentrations and increased exposure levels.

HSD is also examining the responses to pollutant exposures shown by people with compromised immunity, such as people:

- With genetic immunodeficiencies (e.g., severe combined immunodeficiency [SCID])
- Undergoing cancer chemotherapy
- Receiving immunosuppressive drugs (e.g., cyclosporin A) after transplants
- Receiving corticosteroid treatment for various diseases
- With immune-suppressing infections (e.g., acquired immune deficiency syndrome)

Studies with these patients could provide insights into the questions of sensitivity and mechanisms of pollutant action.

People with angina"pain induced by cardiac insufficiency, and the typical symptom of heart disease"are sensitive to carbon monoxide, which affects the time of onset and duration of angina. In the United States, approximately 50 percent of sudden cardiovascular deaths occur in people who have had no previous indication of heart trouble. Many of these people had silent ischemia, i.e., ischemic heart disease without angina. HSD will develop techniques to identify and study individuals with silent ischemia to determine whether they also represent a particularly sensitive population to carbon monoxide. The results of this work will contribute to an understanding of the mechanism of effect of carbon monoxide.

Environmental Epidemiology. Epidemiologic research is conducted primarily on a collaborative or cooperative basis with investigators in different universities, agencies, public health departments, and foreign governments. This provides HSD with the opportunity to enlist investigators with specialized interests, experience, skills, or opportunities to participate in research projects of special interest to the Agency. In addition, because epidemiologic research is very expensive, conducting projects that are jointly sponsored often provides two (or more) organizations the opportunity to conduct research that would have been impossible to a single group.

HSD is co-sponsoring several projects evaluating individual and population responses to air pollution exposures (e.g., the Harvard Six and Twenty-four City Studies, children in summer camps, bladder cancer and chlorinated drinking water) and is very influential in the development of a major research plan to evaluate health effects of chronic exposures to ozone.

International Studies. For several years, HSD investigators have been involved in cooperative studies of lung cancer in relation to indoor coal burning in China. They have also been cooperating with the Chinese EPA in a pilot study of respiratory health (primarily the growth of children's lung function) in several Chinese cities exhibiting a wide gradient of exposure to particulates, sulfur oxides, and acid aerosols. These studies offer the opportunity to determine human health effects across a very wide range of exposures to both indoor and outdoor air pollution. In addition, work has also begun with Thailand's Office of the National Environment Board to launch pilot studies of short-term and long-term human health effects of pesticide exposure and automotive and industrial air pollution exposure in that country. Other studies evaluating the health effects of inhaled particulate matter, particularly acid aerosols, are now possible in Eastern Europe, countries where poor industrial development has resulted in high levels of exposure for the general population. These efforts are expected to provide a rare natural experiment in pesticide use as well as useful information for risk assessment of air pollution.

2.6.3.3 Chemical-Specific Data

Acid Aerosols. To address the need for health effects data on acid aerosols, HSD will conduct chamber studies using controlled exposures to different concentrations of

acid aerosols and measure health effects in the exposed subjects. In addition to pulmonary function, researchers will study the clearance of inhaled insoluble particles because both animal data and some human data indicate that changes occur in the mucociliary clearance rate, depending on the exposure levels. The response of the small airways to acid aerosols will also be tested. Other efforts will investigate the combined effects of acid aerosols and ozone. This clinical research will be complemented by acid aerosol-related measurements, which will be taken in several on-going epidemiologic studies.

UV-B. Because animal data point to UV-B-induced immunosuppression, HSD is evaluating the response of volunteers exposed to UV-B light under controlled conditions. The research focuses on tests for cellular immunity, both at the site of exposure and at a site that was not exposed (systemic immunosuppression). Future work might include efforts to determine whether the degree of skin pigmentation influences the immunosuppressive effect, and whether exposure to UV light diminishes the effectiveness of vaccinations in protecting against infectious disease.

Ozone. HSD is evaluating a major multiyear epidemiologic project to reduce the uncertainty surrounding chronic respiratory effects of recurrent ambient ozone exposure. The study would assess the relationship between ambient oxidant exposure and markers of chronic pulmonary disease, and it might also assess the relationship of ozone exposure to the incidence of respiratory infection and asthma attacks. The results would be exceedingly valuable in reconsidering the NAAQS for ozone.

Drinking Water. To examine the association between drinking water and heart disease, HSD is analyzing the feasibility of conducting an epidemiologic study to investigate the effect of disinfectant parameters and water hardness on serum cholesterol. Other research efforts in drinking water involve comparing the relative importance of different routes of exposure for specific pollutants. Research on indoor air pollutants has shown that, for various volatile organic chemicals such as chloroform and TCE, inhalation is a more important route of exposure than ingestion. HSD is currently studying the extent of volatilization of these chemicals from household use of water. In addition, researchers are conducting a pilot study to determine if human breath analysis can be used to measure radon exposure from the ingestion of drinking water. In the area of disinfection, little is known about the potential human health effects of alternative disinfectants (e.g., ozone, chlorine dioxide, and chloramines) and their by-products. HSD is evaluating the potential for cancer and reproductive effects.

Clusters of adverse reproductive effects have been identified in contaminated water supplies, and animal studies suggest that some compounds found in drinking water may cause these effects at high doses. Epidemiologic research will examine whether contaminated drinking water has reproductive effects.

Alternative Fuels. A major legislative effort has been mounted in the United States to switch from gasoline to alternative fuels such as methanol. The State of California, for example, has already mandated this switch in commercial fleet vehicles. Methanol is a leading candidate as an alternative fuel because the technology for methanol vehicles is well developed and methanol-fueled vehicles are expected to emit lower levels of criteria pollutants than gasoline or diesel vehicles. While the decrease in pollutant levels will very likely benefit public health, very little is known about the potential health effects of methanol, possible additives, and combustion by-products, including formaldehyde. Nearly all the available information on methanol toxicity in humans is related to acute, uncontrolled, high-level exposures, largely by ingestion. Data on human exposure to low levels of methanol vapors are very limited. Clinical and epidemiologic research is needed to determine whether any adverse human health effects are associated with methanol fuel use.

2.6.3.4 Biological Markers

Asbestos and silica are known to cause disease in humans. The function of cells (primarily alveolar macrophages) that have been exposed to these particulate substances *in vitro* is being compared with the function of cells from people who have been environmentally or occupationally exposed to these pollutants. This research is aimed at developing biomarkers to point to the occurrence of potentially harmful exposures before disease develops. Such a tool could contribute to the prevention of environmental diseases.

Other studies will develop and validate both effects and exposure biomarkers. For example, of the almost 1,000 different proteins found in alveolar macrophages obtained by bronchoalveolar lavage, over 100 are consistently and predictably changed following exposure to certain pollutants. This "fingerprint" of response seems to be unique to individual pollutants in exposed individuals. Such protein fingerprints could potentially be used as

biomarkers to indicate that a particular exposure has taken place and also as a tool to find individuals who might be particularly sensitive to a given pollutant.

HSD is also developing tools for detecting potential molecular biomarkers of effect. Researchers are continuing to adapt a molecular biological technique for gene amplification (the polymerize chain reaction technique) to look at messenger RNA in cells of subjects exposed to particular pollutants. This approach, if successful, should enable measurement of the effects of past pollutant exposures on processes and analysis of factors such as gene regulation, growth factors, coagulation factors, immunological and inflammatory mediators, and cytokines.

Cotinine is a metabolic by-product of nicotine metabolism. HSD is developing a kinetic model to facilitate the use of cotinine in urine as a biomarker for measuring children's exposure to secondhand cigarette smoke. The research effort is obtaining data on how rapidly cotinine in urine is cleared in children, so that models for relating cotinine measurements back to levels of cigarette smoke exposure can be developed.

HSD epidemiologists are developing methods for detecting genetic damage in human specimens analyzed in field studies. They are applying assays for detecting modifications at each of the three levels of organization of genetic material"chromosomal, gene- or locus- specific, and chemical (DNA nucleotide). Initially, this work will examine individuals who have relatively high levels of exposure. At a later point, the assays' sensitivity for detecting genetic damage in people with lower, environmental exposure levels will be determined.

Researchers are using molecular assays in a protocol involving occupational exposures. In this study, serial blood samples will be taken from individuals who have experienced relatively high occupational exposures, and their genetic material will be examined through time to determine whether the exposures resulted in biological changes. Positive results would suggest similar studies should be conducted in populations with lower, more environmentally relevant exposures, to determine whether these biomarkers of exposure are also valid at these lower concentrations.

In addition, HSD is measuring the chemical modification of DNA (DNA adducts) in human tissues, such as blood samples, bronchoalveolar lavage, and autopsy tissues, to determine if a relationship exists between the DNA adducts in these tissues and cancer in different organs. Initially, smokers will be selected as the study population because of their relatively high exposure to certain pollutants. These studies will help determine the potential of DNA adducts to serve as markers of biologically significant exposures and effects. In a related study, investigations will determine the relationship between modifications in the DNA adducts and other endpoints for genetic damage, e.g., mutation or chromosomal changes. This work is initially being undertaken in subjects with precisely determined exposures to chemotherapeutic drugs.

Growing concern is focusing on the potential reproductive effects of environmental chemicals. Frequently, members of communities that have been exposed to some form of pollution claim that this exposure has caused an elevated spontaneous abortion rate. Reproductive epidemiology is a relatively new area of research that can help determine whether pollutant exposures are associated with effects such as infertility, pregnancy loss (spontaneous abortions), congenital defects, prematurity, low birth weight, and altered sex ratio.

Many generic issues need to be addressed as reproductive epidemiology matures. For example, measuring the rate of spontaneous abortions with any accuracy is currently difficult. Much research in this area has focused on developing more refined ways of measuring fetal loss, particularly very early fetal loss that occurs before clinical recognition of pregnancy. The development of sophisticated techniques for measuring urine human chorionic gonadotropin"a biomarker for pregnancy"has aided in better defining the baseline rates of spontaneous abortions. Similar research is needed for other reproductive endpoints in order to increase the accuracy and uniformity of these studies.

2.6.3.5 Pollutant Mixtures

Much of the clinical research to date has been related to the effects and mechanisms of response to individual NAAQS pollutants, even though most real-world exposures are to mixtures of pollutants. While some research has been done on pollutant combinations" including sulfur dioxide, nitrogen dioxide, ozone, and several acid aerosols"little is known about the human response to these mixtures. Research is needed to assess whether pollutant combinations cause more severe effects than individual pollutants. One prime candidate for study is ozone in combination with other pollutants: Animal data clearly show that ozone synergizes with other pollutants. A finding of synergism could be very useful in

determining the air quality standards for the chemicals involved.

Epidemiologic studies of the health effects of pollutant mixtures in ambient air are also needed to provide perspective on the significance and health effects of ambient air pollutants. Several industrial areas in the United States have high levels of chemical complexes, involving hundreds of different pollutants. To support this type of research, refinements in epidemiologic methodology, test methods, and biomarkers are needed to determine the important exposures in chemical mixtures and how to measure them.

2.6.4 Emerging Issues

2.6.4.1 Indoor Air

Historically, the focus of much environmental health research has been effects of pollutants found outdoors. In recent years, scientists have increasingly realized that the quality of indoor air plays a major role in human health. Many people spend far more time indoors than outdoors, and pollutants can accumulate to relatively high levels in indoor environments, especially those without adequate ventilation. An ever-increasing number of indoor air pollutants are being identified:

- Combustion products from stoves and fireplaces
- Emissions from carpets, furniture, and building materials
- Microbial organisms that grow in air conditioning systems
- Environmental tobacco smoke
- Radon
- Organics in drinking water that volatilize during household use

Little is known about the health effects of exposure to many of these pollutants. Clinical and epidemiologic studies are needed to determine whether indoor air pollutants pose a significant health risk.

2.6.4.2 Immunotoxicology

Novel and exciting challenges are developing in the field of immunotoxicology as safety assessment issues emerge regarding the use of species-specific recombinant biologicals, biological and biochemical pesticides (e.g., recombinant microbes), and monoclonal antibody reagents designed as drug delivery or detoxification vehicles. Examining these agents requires the thoughtful application of flexible toxicological protocols designed to reveal whether they are immunotoxic, immunopharmacologically active, or immunogenic. The design of such immunotoxicologic studies will be based on an understanding of the potential targets within the immune system and the interactions of chemicals with immunocompetent cells. This approach will rely on new methods that will be developed to examine functional impairment or toxicity in a variety of target organ systems, and on cell and molecular biological techniques that will be more rigorously applied for defining the underlying mechanisms of toxicity. Research is needed to determine whether exposure is occurring and whether any health effects can be measured. This is an area of research of particular relevance to ETD and HSD because inhalation is likely to be the primary route of exposure, with pulmonary immunologic effects as the primary health result.

2.6.5 Summary

2.6.5.1 Hazard Identification

HSD investigators are addressing several tasks that primarily fall under the hazard identification research topic but that also support ongoing dose-response assessment research. For example, HSD epidemiologists routinely and continuously evaluate approaches and develop techniques to estimate exposure and dose to individuals and populations for pollutants of interest. In addition, they play a unique role in exposure assessment by providing design and statistical support to the models used to determine human exposure.

HSD researchers are also developing and evaluating computerized models for use in study design; with these models, they can evaluate the power and specificity of experimental approaches in hypothesis testing.

The development of biomarkers to determine exposure and effects will be facilitated by an integrated approach that incorporates both human and collaborative animal research. This knowledge will be applied in epidemiologic and field studies, which will be used to

evaluate the health impact of exposure to potential environmental contaminants in both water and air. HSD will use *in vitro* techniques to study toxic materials with human tissues and cells to identify potential hazards and to validate animal models. A multidisciplinary approach that addresses endpoints in immunotoxicology, cell biology, and molecular biology will aid in developing and validating both *in vivo* and *in vitro* methods for hazard identification. Techniques for evaluating the public health impacts of drinking water disinfection is one of the pressing research issues facing HSD.

Clinical investigators are developing new techniques to measure dosimetry in humans following inhalation, dermal exposure, or ingestion of a variety of materials, including volatile organic compounds and oxidant gases. HSD is also developing new techniques to measure the growth of inhaled hygroscopic particles in humans. To extend the research to include more sensitive measurements of human health effects, new tests of small airways function are being developed, validated, and incorporated into ongoing studies in which they complement more classical measurements of pulmonary function.

The need to study the human effects of exposure to toxic compounds is pressing, yet exposing volunteers to these substances would be inappropriate. Thus, *in vitro* techniques are being developed that use human tissues and cells. As these approaches mature, resources will be shifted to support their development.

2.6.5.2 Dose-Response Assessment

A major research initiative has been proposed to develop a series of epidemiologic and clinical studies for testing the hypothesis that repeated and continued exposure to ozone, such as occurs in many U.S. urban areas, causes chronic respiratory effects. Because responsive individuals can presently only be identified in clinical exposure studies and long-term exposure to ozone has been shown to produce chronic changes in the lungs of animals, HSD epidemiologists must carefully refer to and integrate clinical and animal studies. As new resources are committed, these investigations will take on increasing prominence in the HSD research program.

HSD has a strong program in dose-response assessment, particularly for NAAQS pollutants. Epidemiologic and clinical research has been used to establish and/or support the NAAQS for ozone, lead, particulate matter, sulfur dioxide, and carbon monoxide. Many important issues remain to be studied for NAAQS pollutants, including identification of sensitive populations, the magnitude of the risk to compromised individuals, determination of the cumulative dose to target organs and sites under differing conditions and durations of exposure, interactions of pollutant combinations, development and validation of new and sensitive tests of response, the mechanism of pollutant action in individuals, and the association between an acute response to pollutant exposure and development of a chronic condition.

SECTION THREE
CURRENT AND FUTURE DIRECTIONS IN HEALTH RESEARCH BY REGULATORY PROGRAM

In this section, current and future research efforts under the EPA health program are categorized first by program office and program, and then by research topic (the x and y axes in Figure 1-2). Sections 3.1-3.6 outline needs and research strategies under the Office of Air and Radiation, Office of Water (Drinking Water), Office of Water (Water Quality), Office of Pesticides and Toxic Substances, Office of Emergency and Remedial Response, and Office of Solid Waste. Program data needs cutting across hazardous identification and dose-response assessment (e.g., biomarkers) are listed under exposure or dose-response assessment. This discussion presents the regulatory point of view on the Agency's current and future health research needs.

3.1 OFFICE OF AIR AND RADIATION

The Clean Air Act (CAA) gives EPA authority to set national standards for ambient air quality and to regulate sources of pollution. The Act explicitly states that the basis for regulation is protection of public health and welfare. In addition to setting standards, the Agency has sought to protect public health from the adverse effects of air pollution by other methods, such as public education, support of state and local regulatory programs, and the banning of products that pollute the air. The foundation of these activities is also the protection of public health or welfare risks.

This discussion of research needs of the Office of Air and Radiation (OAR) first describes the programs supported by the office, outlines each program's issues and research needs relative to the research topics listed in Section One (i.e., hazard identification, dose-response assessment, and exposure), and highlights the health research plans that respond to these program needs. The OHR/HERL research program in support of the Office of Air and Radiation is designed to fill those needs (see Table 3-1).

Table 3-1:

NAAQS, AIR TOXICS, MOBILE SOURCES, INDOOR AIR

Issue	Priority	Needs	Research
Hazard ID	med	Identification of chemicals and endpoints	Methods development
			SAR Clinical methods Short-term noncancer endpoints esp. neurotox, repro/devel, immunotox, pulmonary, genetox/cancer *In vitro* to *in vivo* extrapolation methods
Dose-Response	hi	Estimation of human population risks	Dosimetry/pharmacokinetics
			Extrapolation Species-to-species Route-to-route High-to-low-dose
		Identification of lowest concentration of concern	Sensitive subpopulations
			Mixtures
Exposure	hi	Identify emission sources	Extrapolation across various exposure scenarios
		Identify problem chemicals	Biologic characterization of atmospheric transformation
		Demonstration of cause/ Effect of emissions	Biologic characterization

3.1.1 Office Programs

OAR supports six statutory and programmatic activities with specific health research needs that are addressed by the OHR/HERL research program:

- Ambient Air Quality
- Air Toxics
- Mobile Sources
- Indoor Air
- Global Atmospheric
- Radiation

3.1.1.1 Ambient Air Quality Program

EPA has established National Ambient Air Quality Standards (NAAQS) for six pollutants or classes of pollutants identified in the Clean Air Act: carbon monoxide, fine particulate matter, lead, nitrogen dioxide, ozone, and sulfur dioxides. These pollutants were singled out because they are found ubiquitously and present a substantial danger to public health and welfare. Significant progress has been made in controlling emissions of these pollutants. Great increases, however, in the number of vehicles in recent years have overwhelmed these improvements in many areas. As a consequence, these pollutants remain a major public health issue. State governments, with federal assistance, develop and enforce plans to meet these standards. EPA also establishes standards to restrict emission from new facilities or from facilities undergoing major modifications (New Source Performance Standards). The Agency is required to periodically review the NAAQS standards and to work with state and local air pollution control agencies to achieve progress in complying with these standards.

3.1.1.2 Air Toxics Program

This program has responsibility for all pollutants, other than the six covered by NAAQS, that are potentially damaging to public health or welfare. OAR's strategy for controlling air toxics is three-pronged:

- **Use the authority provided by federal regulation of emissions from stationary sources.** These National Emission Standards for Hazardous Air Pollutant (NESHAPs) control emissions of pollutants that cause "serious irreversible or incapacitating reversible illness." Under this program, regulations have been established for seven pollutants"asbestos, beryllium, mercury, benzene, vinyl chloride, radionuclides, arsenic"and proposed for coke ovens. The Agency has also announced an intent to list nine other chemicals as hazardous air pollutants. Pollutants can also be regulated using New Source Performance Standards (NSPS), which are used to restrict emissions of both NAAQS and specially "designated" pollutants from new industrial facilities or existing facilities undergoing major modifications or reconstruction.

  Under proposed amendments to the CAA, 191 potentially regulated pollutants are identified. Plants emitting greater than 10 tons of any individual pollutant or 25 tons of a mixture of pollutants are subject to Maximum Achievable Control Technology unless the plant can demonstrate it poses a negligible public health risk. It will be up to state agencies to decide whether the risk is negligible, but EPA must provide guidance on how to make this determination. To do this, EPA will identify concentrations or "health benchmarks" that present a negligible health risk for both cancer and for non-cancer endpoints. The Agency must also assess sources of the 191 pollutants for excessive post-control or "residual" risks. Assessments of health hazards will be needed to add or delete chemicals from the list of 191 pollutants.

- **Assess toxic urban air pollution.** Although urban air is known to contain toxic substances, it is difficult to determine which pollutants are hazardous, the effect of exposure to mixtures and atmospherically transformed chemicals on public health, the sources of these emissions, and which chemicals and sources should be regulated. The Office of Air Quality Planning and Standards (OAQPS) has efforts directed at characterizing this problem and determining future regulatory efforts.

- **Provide state/local assistance and high-risk sources programs.** EPA also provides technical assistance to state and local governments. The Agency provides technical support to state and local agencies and EPA's regional offices in implementing air pollution control programs and assists in developing control strategies for toxic emissions, implementing state plans, developing regulations, evaluating operation

and maintenance problems, reviewing new sources to determine appropriate control technology, and evaluating source emission and control strategies. To facilitate these efforts, the Agency has established a technology transfer center. The Control Technology Center (CTC) is a joint effort of the Air and Energy Engineering Research Laboratory in Research Triangle Park, North Carolina; the Center for Environmental Research Information (CERI) in Cincinnati, Ohio; and OAQPS in Research Triangle Park, North Carolina.

An additional technology transfer center is EPA's Air Risk Information Support Center (Air RISC). This joint effort of the Office of Health and Environmental Assessment (OHEA), CERI, and OAQPS assists state and local air pollution control agencies and EPA regional offices with technical matters concerning health, exposure, and risk assessments for toxic air pollutants. Air RISC's primary goal is to serve as a focal point for obtaining information and to provide assistance in the review and interpretation of that information. It provides health and risk assessment information for chemicals being evaluated in the permit review process, assists with on-site risk assessments, and provides guidance on current methods available to conduct health risk analyses.

3.1.1.3 Mobile Sources Program

The CAA requires the Agency to ensure that mobile source emissions from new vehicles, engines, fuels, and fuel additives do not adversely impact health. The Act mandates control of hydrocarbons, carbon monoxide, and nitrogen oxides for light-duty vehicles such as automobiles. In addition, the Agency has developed or is developing emission standards for other types of vehicles. EPA enforces its mobile source standards by an extensive vehicle testing and certification program. The Agency has also promoted the development of state programs for prevention of vehicle tampering and fuel switching. High ozone and carbon monoxide areas are required to have vehicle inspection and maintenance programs. In addition, the CAA authorizes the Agency to regulate motor vehicle fuels and fuel additives in order to protect air quality. The Energy Policy and Conservation Act (1974) and the Alternative Fuels Act (1988) were passed to move the country toward alternative fuel use in order to decrease dependence on foreign oil and facilitate attainment of NAAQS standards in some nonattainment areas of the country. This shift towards the use of alternative fuels has significant implications for health research conducted for the Mobile Sources Program. In addition to the research conducted at HERL, a significant part of the health effects research on mobile source emissions is conducted through the Congressionally mandated Health Effects Institute (HEI). HEI is jointly funded by EPA and the automobile industry and performs research on the health effects of pollutants related to mobile sources.

3.1.1.4 Indoor Air Program

For a number of years, EPA has been addressing the complex issues associated with indoor air pollution via a program of public education and information dissemination. In 1987 Congress passed, as part of a Superfund bill, the Radon Gas and Indoor Air Quality Research Act. This legislation established an indoor air quality research program; directed EPA to coordinate with federal, state, local, and private efforts to improve indoor air quality; and encouraged continued work in information dissemination. The program focuses on the identification, characterization, and monitoring of the sources of indoor air pollutants, human health effects, mitigation measures, and dissemination of information to ensure the public availability of these findings. The Agency has no regulatory authority and, therefore, cannot set enforceable standards for indoor air quality. EPA advisory information, however, may recommend safe levels for specific contaminants. A variety of indoor air pollutants such as tobacco smoke, fumes from combustion appliances, biological contaminants (e.g., molds, mildew, fungi), and volatile organic compounds from building materials are known to have significant adverse health effects and are encompassed in EPA's efforts.

3.1.1.5 Global Atmospheric Program

EPA is directing research into the potential for air pollution to create climatic, ecological, and health problems of global significance. Complex chemical interactions in the earth's atmosphere are now suspected of increasing average temperatures (global warming), changing climatic patterns, depleting stratospheric ozone, and contributing to acid rain. To correct or reverse these trends, EPA is studying the policy options that could stabilize the amount of gases in the atmosphere and control future warming. EPA is also studying the health and environmental effects of potential warming trends. HERL is currently researching the association between the depletion of stratospheric ozone and immune system effects.

Under a Congressional mandate embodied in the Global Climate Protection Act of 1987, EPA is helping to coordinate a national policy on global warming. EPA has

also directed large-scale research into the atmospheric chemistry and long-range atmospheric transport problems of acid rain formation, as well as into methods for restoring lake habitats. EPA also assisted in the development of the Montreal Protocol, an international agreement to reduce consumption of chlorofluorocarbons, halons, and other chemicals that reduce atmospheric ozone. EPA's position was based partly on the results of a 1987 risk assessment of the projected health and environmental effects of reductions in atmospheric ozone. The Montreal Protocol was signed in September 1987 and went into effect January 1, 1989. EPA promulgated regulations (published in the *Federal Register*, August 12, 1988) to implement the phaseout of chlorofluorocarbons, halons, and other chemicals in accordance with the Protocol.

3.1.1.6 Radiation Program

Although radioactive materials fall within the term "air pollutants" in the CAA, the courts found in favor of companies that challenged Minnesota when it used the CAA in an attempt to regulate radioactive materials more stringently than the Atomic Energy Commission. To remedy this situation, an amendment was added to the CAA in 1977, Section 122, that mandated that the EPA Administrator had to review all "relevant information and determine whether or not emissions of radioactive pollutants (including source material, special nuclear material, and byproduct material), cadmium, arsenic, and polycyclic organic matter into the ambient air, ... may reasonably be anticipated to endanger public health." If so, the Administrator must regulate the pollutant under Sections 108, 111, 112, or any combination of these sections.

EPA currently implements a number of programs to protect the public from the health hazards of radiation contamination. While the Department of Energy (DOE) and the Nuclear Regulatory Commission (NRC) have jurisdiction over many facilities that handle radioactive materials, EPA regulates the exposure of the general public to radiation. Virtually all of these regulatory, guidance, and analytical programs are based to some degree on the health effects of radiation exposure.

3.1.2 Office-Specific Research Needs

3.1.2.1 Ambient Air Quality Program

Two factors shape the risk assessment and research needs for the NAAQS program: 1) the limited number of identified pollutants that are regulated by this program; and 2) the widespread exposure to these pollutants, along with the tremendous public health, societal, and economic implications of that exposure. In light of these factors, research needs, by risk assessment step, are as follows.

Hazard Identification. Relative to other programs in the Agency, little work is required to identify new pollutants. Needs in the hazard identification area include confirming associations between specific health effects and identified pollutants, and developing evidence that these health effects are likely to occur in humans. Research efforts developing methods and protocols to address these concerns are ongoing. The focus of this research is generally on very specific questions, such as:

- What are the pulmonary and immune system effects of chronic low-level ozone and/or acid aerosol exposures?

- What are the public health implications of subtle pollutant-related effects observed in animals, such as increased lung membrane permeability ("leaky lung") or increased collagen build-up?

Dose-Response Assessment. As noted above, the pollutants addressed by the NAAQS are relatively unusual in terms of extent of exposure and associated public health risks. As a consequence, risk assessment questions must be answered much more precisely and conclusively than is common for other environmental problems. A central question for NAAQS pollutants is, what percentage of the population, including sensitive subpopulations, is likely to respond at various exposure concentrations? Research efforts to address this question must emphasize human data, animal-to-human extrapolations, and an understanding of mechanisms of action to facilitate interpretation of subtle effects occurring at very low concentrations.

Exposure Assessment. The exposure issues of concern are related to the hazard identification and dose-response questions noted above; for example:

- Can more sensitive biologic indicators of human exposure and resultant effects be developed?

- What are the chronic effects of repeated acute exposures?

3.1.2.2 Air Toxics Program

Two issues significantly shape risk assessment and research needs for this program: the large number of pollutants and sources, and the lack of a substantial database for most pollutants. As a consequence, efforts are focused on improving rapid screening capabilities, as well as developing approaches and assumptions that are valid for many pollutants and mixtures. Although complex issues such as impacts on sensitive human subpopulations are of concern to the Air Toxics Program, research in these areas must generally await resolution of more basic questions.

Research needs in this area, by risk assessment step, are as follows.

Hazard Identification. Regulatory responsibility for many pollutants makes hazard identification one of the most difficult charges of this program. Two of the most pressing questions are:

- What are the chemicals of greatest concern for public health?
- Are the health endpoints associated with these chemicals likely to occur in exposed people?

Dose-Response Assessment. The Air Toxics Program has historically required two types of dose-response assessments: 1) relatively simplistic assessments for preliminary federal regulatory assessments, support of state and local programs, and assessment of urban toxics; and 2) detailed assessments, generally concerning widespread exposures, used in the development of national emission standards (e.g., for benzene cancer risks). The relatively simplistic assessments depend heavily on generic risk assessment procedures and extrapolation assumptions that use pre-existing data. The detailed assessment requires more sophisticated analysis and detailed data, similar to that required for the NAAQS pollutants; new data and methodologies must often be generated to support the assessment. Use of these two levels of assessment will likely continue in making negligible risk determinations and residual risk determinations.

Important questions for both simplistic and detailed assessments include:

- How can risks be assessed for large numbers of pollutants based on relatively little data?
- How should risks from multipollutant sources be assessed?

To answer these questions, research into rapid screening and evaluation techniques and improved route-to-route, species-to-species, and low-dose extrapolation methods"as well as into the underlying issues of target dose and mechanisms of action"must be performed. Equally critical is discerning adverse critical effects for each target system, the interactions between toxic events at different organs, and the progression of toxicity, particularly for toxics where multiple noncancer events are possible. HERL's role is to develop data to support these generic approaches and to develop effective assessment tools, e.g., quantitative methods for noncancer health effects.

Exposure Assessment. The need to characterize emissions from a variety of sources for many chemicals results in several questions for research efforts:

- What are the most significant sources of pollution?
- Can resulting exposures be demonstrated to be causally related to adverse health effects?
- Are nontoxic chemicals atmospherically transformed into toxic products?

3.1.2.3 Mobile Sources Program

Historically, the statutory and programmatic issues that shape mobile source-related research were very similar to NAAQS: a limited number of classes of pollutants of concern and enormous potential public health impacts. However, the needs of this program have broadened over time. Research is now needed to develop methods and data to identify pollutants of concern and assess the health effects of currently unregulated pollutants and complex mixtures associated with mobile sources. Needs center on identifying the health effects of certain pollutants or mixtures"for example, diesel exhaust"and on gathering detailed information on dose-response relationships, especially on extrapolation to humans, including sensitive human subpopulations. The Agency is committed to analyzing the relative risks of alternative fuels to enable development of a national alternative fuels strategy. It is anticipated that the research requirements for such a comparative assessment, which are discussed in the Agency's Alternative Fuels Research Strategy, will receive considerable attention.

3.1.2.4 Indoor Air Program

The risk assessment questions for indoor air are similar to those for air toxics, probably due to the programs' similar responsibilities for many pollutants and a variety of sources. In some areas, however, the Indoor Air Program has some special interests:

- What are the sensitive indicators of toxicity in readily identifiable subpopulations"for example, the "ill""who are likely to spend all of their time indoors and are thus at greater risk from indoor pollutants?
- What pollutants are harmful and at what concentrations?
- Can chemically sensitive or hypersensitive populations be characterized relative to indoor air pollution problems?

3.1.2.5 Global Atmospheric Program

Global atmospheric issues are still emerging. The most pressing health issue identified so far is associated with ultraviolet (UV) radiation. Due to the potential for tremendous public health impacts, research on the health effects resulting from increased UV-B exposure and associated dose-response relationships has been given a high priority by HERL. UV appears to have complex effects on the immune system; at least in mice, these effects appear to induce the generation of T-lymphocytes that suppress immune responses in a long-lasting and specific manner. Whether increasing UV radiation at the earth's surface will cause immune suppression, thus leading to increased population susceptibility to infectious agents, remains a distinct possibility. No studies in humans has yet addressed whether and how UV exposure alters human immune response. Questions of concern to the program office include:

- Does UV light exposure in humans result in systemic immunotoxic effects?
- If so, are the effects long-lasting and specific?
- Can the effects be demonstrated using *in vitro* assays?
- What are the mechanisms of the effects?

3.1.2.6 Radiation Program

Research is also needed to elucidate the mechanisms and dose-response relationships associated with exposure to radiation. An area of emerging interest is electromagnetic radiation (EMR). Effects have been reported on the brain, the reproductive system, and the immune system. EPA is currently developing a research strategy in this area.

3.1.3 Research Plan

Proposed air research will address the questions of concern to the program offices. The research areas identified here are critical to support risk assessment across programs. Key research areas common to each program's needs include clinical and animal inhalation toxicology and epidemiology. Work under the research topic of hazard identification will focus on method developments that will enable identification of agents and endpoints of concern. Efforts will be included to determine the relevance of these endpoints to the human population. Dose-response work will focus on dosimetry, pharmacokinetics, and mechanisms of action as key areas in understanding target dose, and a variety of extrapolation questions. The aim of this research is the improvement of the ability to estimate human population responses to exposure, based on animal and human data for a variety of exposure scenarios (e.g., intermittent vs. chronic exposures). Exposure research will use biologic endpoints to characterize exposure and to link exposure to effects. This work will result in more effective identification of sources and agents of concern as well as strengthen cause-and-effect relationships between emissions and human health effects. Taken together, these research efforts will ensure the credible and effective use of risk assessment in Agency decisions.

3.1.3.1 Ambient Air Quality Program

Hazard Identification. Research will focus on the development of more sensitive and specific bioassays to identify more subtle indices of damage for known effects and facilitating the interpretation of the significance of these effects for public health. Understanding of mechanisms of action of toxic agents and of disease processes is an important part of this effort. Two areas of this research effort are particularly noteworthy:

- **Understanding the impacts of chemical exposure on the immune system.** Available data suggest that substantial impacts on the immune response may occur as a result of low-level pollutant exposure and that immunotoxic effects are one of the most sensitive indicators of toxicity. Currently, the nature of the immune response is characterized using responses in lung and host-resistance models. A major goal of this research is to correlate the effects of chemical exposure on immune function test with effects that are seen in the human population such as susceptibility to infections, allergic reactions, and autoimmune disease.

- **Identifying subtle indices of pulmonary damage that are believed to be the early hallmarks of serious respiratory disease.** For example, several lines of animal and epidemiologic evidence indicate that many airborne pollutants can damage small airways. This damage cannot be detected clinically. Sensitive clinical techniques need to be developed to allow acquisition of human data in this critical area. As noted above, work in this area is designed to develop sensitive test methods that will allow comparisons between human and animal as well as between *in vivo* and *in vitro* data.

Dose-Response Assessment. Research under this topic will allow risk assessors to accurately estimate population responses to pollutant exposures. Efforts will fall in three important areas: understanding the mechanism of response and its quantitative relationship to dose, improving extrapolation from animal to human response, and identifying sensitive subpopulations. A key aspect of this research is joint efforts in human and animal toxicology"a research design that is uniquely available at HERL. Animal models of cardiopulmonary and immune system diseases are being developed in animals and validated with both *in vivo* and *in vitro* clinical data and epidemiologic data from humans. Refinement of dosimetric and pharmacokinetic dose-response models is also essential to this research effort. These models enable the accurate estimates of critical dose to target organs that is necessary for risk assessment and extrapolation across dose, species, and route of exposure. Also, dosimetrics and pharmacokinetics, coupled with differences in tissue sensitivity, are the factors that are most likely responsible for differences in population sensitivity. Both human (clinical and epidemiologic) and animal dose-response studies, as well as mathematical modeling, will be given special attention to determine the deposition, clearance, and pulmonary function effects of particles, alone and in combination with ozone, NO_2, and SO_2.

Exposure Assessment. Sensitive biological measures of pollutant damage identified as part of hazard identification and dose-response work will be used in addressing exposure-related issues. Animal and clinical studies examining the effects of repeated acute exposures will be coupled to epidemiologic and/or mechanistic work that will address chronic implications for human health. The use of biomarkers is expected to greatly improve both clinical and epidemiologic estimates of exposure and effects. These biomarkers, in conjunction with animal experiments, are expected to play a critical role in answering questions about the effects of ozone, nitrogen dioxide, and acid aerosol mixtures.

3.1.3.2 Air Toxics Program

Hazard Identification. As an aid in identifying numerous chemicals and endpoints of concern, HERL is developing methods for a tiered approach to screening and characterizing pollutants. Current tests are often expensive and time-consuming and do not identify all important effects. Work in this area will be directed at developing more rapid and accurate assessments.

The initial toxicity screening could be performed based on structure activity and *in vitro* test data. Subsequent work will validate health concerns identified in the initial screening assessments as well as their relevance to humans. Researchers will focus on expanding techniques that have been developed to assess cancer to a wider variety of chemicals and chemical classes and to develop new tests for noncancer endpoints. Examples of tests that have been developed at HERL and are currently being validated or improved are a genctoxic screen for reproductive hazards; bioassay-directed fractionation; a structure-activity computer program; the Functional Observational Battery, which measures chemical-related effects on sensory, motor, and autonomic nervous system function; and the Chernoff/Kavlock screen for developmental effects. In addition, the research program to support the urban toxic program involves considerable effort to identify complex mixtures of concern, including those created by atmospheric transformation.

Dose-Response Assessment. Of equivalent concern to the Air Toxics Program is dose-response assessment to evaluate levels of risks. Research under this topic will improve understanding of dosimetry, pharmacokinetics, and mechanisms of action and, as a consequence, the dose-response relationship and species-to-species, route-to-route, high-to-low-dose extrapolation issues. The major goal of this research program is to provide better methods

for extrapolating animal data to human effects. Both human and animal experiments will provide data on the functional, morphological, and biochemical changes that occur following exposures to air pollutants; provide extrapolation techniques to predict human responses; and provide data to determine the extent to which air pollutants cause or exacerbate the development of chronic disease. Biological endpoints to be examined include development of cardiovascular or pulmonary disease, impairment of immune function, reproductive and developmental endpoints, and neurotoxicity, as well as liver and kidney dysfunction and cancer.

This information will significantly improve or permit risk assessments of greater certainty for a much larger number of chemicals and exposure situations than is currently possible. Some of the more immediate benefits to the program are likely to be in estimating risks associated with doses exceeding the reference dose, reduction or elimination of some uncertainty factors, and gathering information concerning appropriate methods to use when assessing the risk associated with chemical mixtures. The health effects data from this research program will be incorporated into EPA documents to support regulations, to define problems, to assist state and local agencies, and to evaluate high-risk point sources.

Exposure Assessment. Sensitive biologic measures of pollutant exposure that will be developed as part of the hazard identification and dose response work should greatly improve estimates of exposure. Work in this area is focused on three issues:

- **Identification of major sources of air pollution using biologic screening.** This use of mutagen assays to screen for significant sources of emissions was developed at HERL. Future work will develop methods for more accurately characterizing and identifying new sources of pollution and evaluation of noncancer as well as cancer health concerns.

- **Identification of relevant atmospheric transformation products.** The development and improvement of similar biologic methods to characterize atmospheric transformation products is also ongoing. Data being developed may be critical in regulation of chemicals that in themselves are innocuous but are transformed in the atmosphere to potent carcinogens.

- **Use of biomarkers of exposure.** Animal testing of DNA adducts as biomarkers of exposure is currently ongoing. Field testing of these biomarkers, as indicators of human exposure, is planned for the near future. Research into the biologic link between these markers and actual cancer risk is also needed. Successful results will make possible direct estimates of human exposure to carcinogens and will provide relatively rapid and inexpensive alternatives to traditional epidemiologic approaches. Significant improvements in such areas as urban cancer risk and improved cause-and-effect evidence for NESHAPs candidate pollutants will also be possible. Biomarkers for pulmonary, immune, and reproductive system effects are under development.

3.1.3.3 Mobile Sources Program

Hazard Identification, Dose-Response, Exposure. Research is currently ongoing on the mutagenicity and carcinogenicity of existing mobile source-related emissions. Studies conducted by ORD have shown that motor vehicles are a major source of risk in urban areas. Research in this area is focused on developing and improving methods for screening and characterizing carcinogenic potential using short-term bioassays. Researchers will also begin to assess noncancer endpoints, particularly developmental and reproductive toxicity, immunotoxicity, hepatic toxicity, respiratory toxicity, and neurotoxicity. Components of mobile source emissions will be identified and the relationship of these assays to relative mutagenic and carcinogenic potencies determined. Dose-response assessment for endpoints of concern will also be evaluated through animal-to-man extrapolation and estimates of sensitive subpopulations. The program also needs human data on ozone, oxides of nitrogen, sulfur dioxide, carbon dioxide, methanol, and aldehydes.

In addition, the health research program plans to collect the data needed to perform a comparative risk assessment of alternative fuels. To do this, the mobile source program needs to establish bioassay and animal testing protocols (as mandated in Section 211 of the CAA) so that it can evaluate the health impacts of new fuels and fuel additives. In addition, the mobile source program needs substantial health research information on methanol and other alternative fuels. For each alternative fuel, data will be gathered to speciate emissions, identify and quantify components, determine variance with the weather and car performance characteristics, and evaluate possible public health consequences. Since much of this information is not available for gasoline, additional research may be undertaken for this fuel as well.

3.1.3.4 Indoor Air Program

Hazard Identification. Research will continue to identify agents common in indoor air that produce adverse health effects. Identifying and interpreting subtle human health effects associated with identified indoor air pollutants will be a key research area. Characterizing sensitive human subpopulations and their responses to indoor pollutants is a major part of this effort. To perform this task, the development of new methods and approaches to the types of effects caused by indoor pollutants will be necessary.

Dose-Response and Exposure Assessment. The research in this area will be directed to the indoor agents of greatest concern, i.e., volatile organic compound (VOC) mixtures and environmental tobacco smoke. Human effects and dose-response of noncarcinogenic VOCs will be determined in relation to the sick building syndrome. Cancer, impaired immune function, impaired lung function, and neurotoxicity are the current focus of this program. Future efforts will expand into other areas, such as reproductive/developmental risks. Bioassay of organics from indoor sources will be conducted in chambers, test homes, and field studies to provide comparative estimates of potential health risks from various sources. Biomarkers of exposure will also be validated to enable demonstration of cause-and-effect relationships in epidemiologic studies.

3.1.3.5 Global Atmospheric Program

Hazard Identification. Research will determine whether immunotoxic effects observed in mice in response to low-level UV-B radiation also occur in humans. *In vitro* methods to study these effects will also be developed.

Dose-Response Assessment. Initial efforts will focus on emulating the UV dosage schedule that is observed to cause immunotoxic effects in mice and is appropriate for human use. Both local and systemic immune responses will be observed. The time course of responses will also be determined. Work will emphasize analysis of the mechanisms of these changes to enable understanding of the potential impacts on human populations, given worldwide exposure.

3.1.3.6 Radiation Program

As already noted, EPA is developing a research strategy in this area. Projects have been proposed to study the connection between immunosuppression from UV irradiation and pathogenesis of infections diseases; the changes in innate and acquired defense mechanisms in mice exposed to radiation; the effects of UV-B on pathogenic and protective immune responses in murine cutaneous leishmaniasis; the influence of UV-B irradiation on the effectiveness of immunization; and the effects of UV-B radiation on susceptibility to murine cytomegalovirus and influenza virus using experimental mouse models.

3.2 OFFICE OF WATER (DRINKING WATER)

The Safe Drinking Water Act of 1974 (SDWA), as amended in 1986, mandates that the EPA ensure a high quality for the nation's drinking water. The focus of the law, and of the regulations developed by the Office of Drinking Water (ODW), is the protection of human health. Public water supplies must be disinfected to prevent pestilence from waterborne infectious disease. Controls are required on the type and amount of disinfectants and other water treatment chemicals in drinking water because these chemicals form by-products after reaction with naturally occurring chemical substances in water. These by-products have biologic activity and the potential to cause adverse human health effects. Thus, regulatory decisions about water treatment requirements involve balancing the risks from infectious disease in raw water and the risks from chemical toxicity in disinfected water. This balancing is achieved through two levels of drinking water standards. The primary drinking water standards limit the amount of microbes and certain chemicals allowed in drinking water. The secondary drinking water standards, which regulate parameters related to the aesthetics of drinking water quality, are not covered here.

The Agency must set Maximum Contaminant Level Goals (MCLGs) and Maximum Contaminant Levels (MCLs) for microbes and each chemical or chemical class to be regulated. MCLGs establish levels in drinking water that are not expected to result in any adverse human health effect over a lifetime of exposure. Based on health effects concerns only, they are not enforceable standards but represent goals that water treatment operators should strive to reach. MCLs are enforceable standards based on health effects concerns, but they also take practical considera-

tions into account (e.g., the technological feasibility of control, availability of analytical techniques and their detection limits, and the economic impact of regulating the contaminant).

MCLs and MCLGs are based on cancer risk assessments or noncancer risk reference dose calculations described in Drinking Water Quality Criteria documents. These documents outline the available scientific database and the risk assessment logic used to derive the numbers. For chemicals that are not ubiquitous in U.S. water supplies but that may enter drinking water by accident, the Office of Drinking Water prepares Health Advisory Documents (HADs). While shorter than the criteria documents, HADs contain similar risk assessment information; they derive One-day, Ten-day, Longer-term, and Lifetime Health Advisories (HAs), i.e., concentrations of a contaminant in drinking water that are not expected to cause any adverse noncarcinogenic human health effects after exposures varying from one day to a lifetime.

The 1986 SDWA Amendments establish a time frame for completing primary drinking water regulations on 83 substances identified by Congress (provisions were also made for adding 25 substances every three years afterwards). The Amendments require disinfection of all public water supplies. EPA is currently evaluating which treatment combinations are the most effective for microbiological control and produce the least noxious chemicals. Regulatory options include:

- Requiring removal of organic materials from raw water prior to disinfection to eliminate the precursors of disinfection by-products
- Weighing the various primary and residual disinfection possibilities
- Filtrating
- Setting requirements for other modifiers of water characteristics (e.g., pH, alkalinity)

Health research is needed to support ODW's decisions in these areas:

- Placing substances on the list for regulation
- Setting MCLGs and HAs
- Writing Criteria and Health Advisory Documents
- Establishing the basis for
  - Treatment technologies
  - Monitoring
  - Public notification
  - Evaluating efficacy of control technologies
  - Determining risk reduction achieved by regulation

The OHR/HERL research program in support of ODW is designed to fill those needs (see Table 3-2).

Table 3-2:
SUMMARY OF DRINKING WATER HEALTH RESEARCH

Topic	Source Water	Disinfectants and Disinfectant By-Products	Nondisinfectant Additives and Distribution System Contaminants
Hazard ID		Databases and SAR; methods development (e.g., bioassays, reproductive tests)	
Dose-Response	Species-to-species/ organ-to-organ extrapolation; mechanistic studies to develop alternatives to RfD	Pollutant mixtures; species-to-species extrapolation; PB-PK studies	PB-PK studies on metals, route-to-route extrapolation
Chemical-Specific	As part of other studies with focus on metals	Human and experimental animal studies of major disinfectants and their alternates	As part of other studies with focus on metals
Exposure		Uptake and distribution of disinfectants	Uptake and distribution of metals

3.2.1 Office-Specific Research Needs

The key issues that face ODW in its mission to ensure a safe, high-quality drinking water supply are:

- Microbiological and chemical quality of source waters
- Safety of disinfectant processes
- Impact of nondisinfectant chemical additives (e.g., clarifying agents) on human health
- Safety of corrosion by-products and chemicals that might leach from the water distribution system prior to arrival at the tap

These issues are discussed in this section under three topic areas: 1) source water; 2) water disinfection and disinfection by-products; and 3) nondisinfectant additives and distribution system contaminants. These topics are not mutually exclusive. Points that apply to more than one area will be made under one topic only.

3.2.1.1 Source Water

Drinking water comes from either surface (e.g., rivers, lakes) or ground waters (aquifers). The water from these different sources varies greatly in pH, hardness and other mineral characteristics, microbiological and chemical contamination, and organic material (e.g., leaf mold). Ground waters are generally of better quality than surface waters, with fewer microbes, little organic material, and fewer pollutants. These waters, however, may contain higher concentrations of chemical substances, such as pesticides in agricultural areas or solvents from underground injection of wastes.

These characteristics"which greatly impact the quality of the treated water"must be considered in choosing treatment technologies. For instance, raising pH lowers the leaching of lead from pipes and solder in the distribution system and also lowers the formation of choloroacetic acids in chlorinated water; at the same time, however, it increases the formation of trihalomethanes, which include known human toxicants like chloroform. In addition, removal of organic material lessens the formation of hazardous disinfectant by-products in treated water, and reducing water hardness lowers the corrosivity of the water reaching distribution pipes; but both these options add a significant cost to finished water and neither is absolutely achievable.

ODW and water suppliers must ask:

- What chemicals are present in a given source of water?
- Which have adverse health effects?
- Of these, what is the basis for deciding to remove the substance from drinking water or reduce its amount in water?
- What detection and monitoring techniques are available?
- What is the cost of monitoring and control options?
- What is the minimum amount causing an effect?
- How should health effects concerns be balanced with monitoring and control technology to determine how drinking water contaminants should be regulated?

Research in this area should focus on providing the scientific basis for setting MCLs and developing HAs. For most of the metals (e.g., nickel, chromium) and organic pollutants (e.g., pesticides, solvents) present in source water, data are available concerning hazard potential. These data are often generated to meet the mandate of other environmental laws such as the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) or the Toxic Substances Control Act (TSCA). (For information on organic materials in source water, see Section 3.2.2.1; on chemicals responsible for water hardness and corrosion by-products, see Section 3.2.2.3.) To compare the hazard potential of exposure to these materials in source water versus through other media, pharmacokinetic and pharmacodynamic data from the various media must be compared; thus, the prime focus of HERL research for source water concerns will be on dose-response research.

3.2.1.2 Water Disinfection and Disinfection By-Products

Drinking water disinfection has been practiced for many years"especially disinfection of surface waters, which are more heavily impacted by human activity than are ground waters. Disinfectants are strong oxidants, highly reactive with biologic material. These charac-

teristics allow them to sterilize the pathogenic microbes in source water. The more reactive the disinfectant, the greater the immediate disinfectant property, but the less the stability. Because water is not consumed immediately after disinfection, microbes that escape treatment or that are introduced into the distribution system after treatment are a potential health hazard. As a result, secondary disinfection is often employed via residual disinfectant to ensure the microbial safety of water reaching the tap. ODW is seeking ways to reduce the formation of disinfection by-products by offering water suppliers options such as precursor removal prior to disinfection and various treatment combinations, such as ozonation (which does not produce chlorinated by-products) followed by chloramination (to provide a stable residual disinfectant) while still maintaining adequate water safety in the development of its regulations. This process is very complex, and there is much uncertainty surrounding what chemical substances are formed by water treatment, the nature of their health effects, and the choice of the best control technologies.

In evaluating the basis for disinfection requirements, by-product control, and variances, ODW must ask:

- What compounds are formed by the various disinfectant treatments?
- What is their ability to cause human health effects?
- Which results in the lowest chemical hazard potential with the greatest disinfectant properties?
- What treatment technologies are available to achieve the desirable characteristics?
- What detection methods and approaches are available to monitor the effectiveness of treatment technologies?
- What is the maximum amount(s) of disinfectant(s) causing an adverse health effect?
- How should the health effects concerns be balanced with monitoring and control technology capabilities to determine disinfection requirements?

The research focus in this area must be on developing the science base to support water disinfection decision-making. Human data are needed to identify or confirm health hazards associated with alternate disinfectant treatments (e.g., ozone, chloramine, chlorine dioxide) and susceptible subpopulations (e.g., pregnant women and glucose-6-phosphate dehydrogenase deficient persons) exposed to strong oxidants. Extrapolation studies that evaluate high-to-low-dose relationships need to be conducted to evaluate the risks that are likely to occur at the levels of chemicals encountered in drinking water. These studies are key to ensuring safety without undue economic hardship. The disinfectant regulations will cost the water industry an estimated additional $1-2 billion per year, or from 10-20 percent of their current annual income.

Work is also needed on the bioavailability and distribution of disinfectants and their by-products (e.g., ozone and chloramine by-products) from water versus other sources of exposure. Research to develop biomarkers for sensitive endpoints related to disease outcomes (e.g., liver enzymes and the threshold mechanism for liver carcinogenicity of dichloroacetic acid) also is needed. Research efforts should be designed to understand the artifacts induced at high exposure levels that have no bearing on health effects at doses humans receive.

To support risk assessment, pollutant mixture studies are needed to identify the biology of disinfectants and by-products present in both simple and complex combinations. Sample fractionation followed by bioassay will also be used to identify biologically active chemicals as a basis for identifying toxic components and for directing control technologies. Test methods need to be developed for evaluating the structure-activity relationships (SAR) within families of by-products (e.g., trihalomethanes, chloroacetic acids, furanones) to compare potencies between different endpoints (e.g., genotoxicity/cancer compared to reproductive, neurotoxic, cardiovascular, and other organ effects). Databases are needed to systematically store the SAR information for subsequent use in model development, to address adversity/severity of effect, and to select the most appropriate test regimens for untested compounds. The ultimate goal is the development of predictive risk assessment models.

3.2.1.3 Nondisinfectant Additives and Distribution System Contaminants

In addition to chemical disinfectants, other chemical substances are added to drinking water at treatment plants. Clarifying agents such as alum are used to remove particulates suspended in the incoming water, and other substances may be added to adjust such characteristics as pH and water hardness. As drinking water leaves the treatment plant, it passes through distribution mains to connections with various buildings and finally into internal building

plumbing before reaching the tap. As it passes through the system, copper, lead, and other metals may leach into it"for example, from the piping or solder"before the water reaches the tap. Recent regulations limit the lead allowable in new pipes and solders, though pipes and solders in existing structures are not yet regulated. Little is known, however, about the toxicity of metals (e.g., antimony) used to replace lead in solders. ODW must make sure that water entering the distribution system does not contain harmful additives, and that once in the pipes, it does not cause corrosion and leaching of toxic metals.

ODW must set MCLGs and develop HAs for drinking water additives and distribution system contaminants; to do so, it needs research data characterizing the uptake of heavy metals (e.g., nickel, aluminum, copper) and their distribution and fate in the body. Human studies are needed to evaluate the ability of the corrosion by-products to cause reproductive effects and other adverse health outcomes.

3.2.2 Research Plan

For regulatory decision making, information is needed about the uptake and distribution of xenobiotics within the human body, and effects likely to occur at the low concentrations encountered in the environment. Unique ODW research needs result from the regulatory mandate for the Office to make sure source water, disinfection treatments, and distribution systems are consistent with safe, high-quality water.

3.2.2.1 Source Water

Dose-Response and Exposure Assessment. Most information about the chemicals present in source water will be gleaned from the literature or be derived from work conducted in support of other Agency programs: the substances of concern in source waters are largely the pesticides and industrial chemicals used in commerce and metals leaching from the soil through which the water passes. In the water program, the potential for adverse human health effects must generally be based on data from high-dose exposures in whole animals or *in vitro* tests. Questions often arise concerning the uptake, bioavailability, and tissue distribution of materials from source water; thus pharmacokinetic studies are needed to interpret and evaluate data, including:

- Fate and disposition of metals (e.g., arsenic, cadmium, lead) as related to ingestion via drinking water, and route-to-route extrapolation
- Human epidemiologic studies on populations impacted by high metal content (e.g., arsenic) to relate exposure to outcome
- Extrapolation of effects observed at high doses compared to those observed at low doses
- Extrapolation of data from animal studies to humans

A variety of endpoints have been used by ODW to set MCLs in the regulation of drinking water contaminants: nervous system effects, liver toxicity, tumor formation, kidney toxicity, circulatory/heart/blood effects, lung toxicity, reproductive/developmental effects, and gastrointestinal tract effects. To increase knowledge concerning the shape of the dose-response relationships for these substances:

- Work will be conducted to understand homologous endpoints between human and experimental animals for noncancer endpoints, especially for reproductive/developmental effects and neurotoxicity.
- Emphasis will be placed on identifying species-specific and cross-species parameters to support physiologically based (PB-PK) model development.
- The relationships and time dependency between exposure, delivered dose, and outcome will be studied to develop biologic markers of dose and early effect.
- The markers will be used to understand the shape of dose-response curves and the data will be transferred to the Office of Health and Environmental Assessment (OHEA) and other risk assessment groups for application in risk assessment modeling to supplant the use of safety factors in setting RfDs.

Chemical-specific data are needed because ODW cannot require industry to test chemicals. However, many of these chemicals are of interest to more than one program office. For instance, ODW and the Office of Solid Waste and Emergency Response (OSWER) share an interest in the effects of exposure to metals. HERL will conduct research using chemicals of interest to ODW, especially metals (e.g., arsenic, nickel, chromium, cadmium).

3.2.2.2 Water Disinfection and Disinfection By-Products

In relation to disinfectants and disinfectant by-products, HERL researchers will address fundamental questions about the safety of oxidants at the low doses encountered in drinking water and will provide data for comparing various disinfection treatment options in regulatory decision making. HERL will give research in this area high priority because most of the chemicals of concern (i.e., the disinfectant by-products) are formed inadvertently and ODW cannot require industry to test them.

Hazard Identification. Thousands of chemical substances are formed through chemical reactions between disinfectants and organic material present in source water. Few data exist concerning biologic activity associated with exposure to these substances. Because testing them would be prohibitively expensive, as well as impractical, substantial efforts are needed to develop SAR as well as to develop, validate, and refine test methods and testing strategies for evaluating the hazard potential of halogenated disinfectants and their by-products, alone and in mixtures.

These efforts include:

- Developing databases
- Using the databases in risk assessment and the development and refinement of SAR models and techniques
- Constructing graphic displays of a variety of endpoints, enabling comparisons of toxicity by class of compound, outcome, and potency as a basis for biologic SAR
- Using SAR and profiles for generating hypotheses, developing testing strategies, and prioritizing research compounds

Dose-Response and Exposure Assessment. Some treatments being considered as alternative technologies that might replace chlorination, such as ozonation and chloramination, have been used for several decades or more in certain communities in the United States and abroad. A major uncertainty surrounding these chemicals is whether the treatments and their by-products are as hazardous or perhaps more hazardous than those resulting from chlorination.

Animal studies. HERL researchers will perform studies on experimental animals to provide a basis for assessing human risk. Most emphasis will be placed on evaluating the hazard potential for disinfectants other than chlorine"especially for ozone and chloramine, which have been identified as potential candidates for alternative disinfection. Stoichiometric differences in the half-lives and by-product formation of these and other disinfectants (e.g., chlorine, chlorine dioxide) in the gut will be modeled as a basis for predicting outcomes from high-dose experimental to low-dose environmental exposures and for designing follow-up toxicological studies. To the extent feasible, the models will be used to predict toxicity and follow-up pharmacokinetic and pharmacodynamic studies will be conducted to verify the predictions.

Such studies will form the groundwork for targeting subsequent research efforts to identify the presence and nature of artifacts that may be induced by high-dose regimens but are not an issue with low-dose environmental exposures. The work will also be used in developing biomarkers of organ dose and early biologic effect to define the shape of the dose-response curves for various adverse outcomes. This information will be useful in developing the quantitative risk assessment models that will eventually replace RfDs.

These efforts will focus on developmental effects, cancer, and liver toxicity. The carcinogenicity studies will emphasize the development of parameters for the Moolgavkar-Knudson-Venzon model and the elaboration of dose-response curves for nongenotoxic carcinogens as a basis for exploring the possibility of threshold mechanisms of cancer induction. Developmental effects studies will employ the techniques of cell biology and flow cytometry to elucidate early events leading to stage- and species-specific alterations in embryonic development. Similarly, studies on liver metabolism will focus on early biochemical changes of organ function for use in elucidating the dose-response. To the extent possible, these studies will be conducted in parallel to provide comparative toxicity information.

Human studies. Human data will also be collected to evaluate increases in morbidity and mortality linked to the consumption of ozonated, chloraminated, and ozonated/chloraminated water compared to the consumption of chlorinated water. As appropriate, similar comparisons will also be made for persons consuming water treated with chlorine dioxide and other possible alternative disinfectants, if suitable populations can be found. Studies on persons consuming ozonated water will probably be conducted in Europe, which has the longest history of use.

However, care will have to be taken to select communities that employ treatment processes similar to those likely to be used in the United States. Studies of populations consuming chloraminated water will probably be conducted in New Orleans, Louisiana.

Studies of mixtures. Because drinking water is a complex mixture of chemical substances, the health research program will focus on evaluating the effects of disinfectants and their by-products after combined rather than single exposures. The two thrusts of this effort are:

- To understand principles and develop databases for risk assessment
- To identify the toxic species within mixtures so that control technologies can be developed to eliminate or reduce them from potable water

The current emphasis in these studies is on the application of short-term tests for genotoxicity in combination with chemical fractionation. While this approach has proven successful in identifying genotoxic compounds in drinking water"for instance, in the identification of hydroxyfuranones (e.g., MX) as potent genotoxicants"the relationship between a chemical's potency as a genotoxicant and its potency for other toxic effects is unknown. Short-term test methods will be developed for reproductive and developmental effects, liver, and, if possible, neurotoxic outcomes; these will be applied to model compounds, simple mixtures, and then to drinking water samples. The data will be used to assess the toxicity of water treated with a variety of disinfectants.

3.2.2.3 Nondisinfectant Additives and Distribution System Contaminants

The pollutants used as additives or that arise from the distribution system and contribute to the degradation of water are primarily metals: aluminum used as a clarifying agent; copper, lead, and antimony from water pipes, joints, and solder. The primary target organs for these substances are the nervous and reproductive systems and the kidney.

Dose-Response and Exposure Assessment. Research efforts in this area will focus on these target organs, concentrating on the uptake and distribution of metals"primarily nickel, aluminum, and copper from the gastrointestinal tract"and their transport to the reproductive and nervous systems. Studies will be conducted under both high-dose and more realistic environmental conditions as a basis for extrapolating from high to low dose and to compare uptake and response from different routes of exposure.

Studies of nonconventional, lifetime exposure (e.g., effects over two and three pregnancies), and susceptible life stages (e.g., very young, very old, pregnant) will be conducted using experimental animals to approximate human conditions not modeled by conventional assays. Researchers will also perform human studies to refine the results of the animal studies for risk assessment use.

3.3 OFFICE OF WATER (WATER QUALITY)

The Federal Water Pollution Control Act of 1972, as amended by the Clean Water Acts (CWA) of 1977, 1978, 1980, 1981, and 1987, require that the quality of the nation's surface water supplies be maintained for their intended purposes (i.e., that they be swimmable, fishable and/or navigable). The states are responsible for defining the uses for which surface waters are intended, issuing permits, and monitoring discharges from industrial and wastewater treatment sources to ensure that water quality is maintained.

All sources and types of pollution of surface waters (i.e., rivers, streams, lakes, bays, estuaries, most natural wetlands and oceans) are covered by the Water Quality Program. EPA regulations address "conventional pollutants" (i.e., biological oxygen demand, suspended solids, fecal coliform, pH, and oil and grease), and toxic pollutants (i.e., 65 classes of chemical substances listed in the CWA). From these 65 classes of toxic pollutants, EPA has identified 126 compounds as priority pollutants. In addition to the conventional and toxic pollutants, EPA typically considers another 300 or so pollutants for regulation that are known to have adverse effects and for which accurate measurement techniques have been developed. These are referred to as nonconventional pollutants.

EPA must publish water quality criteria for the priority pollutants that set forth the maximum concentrations consistent with the goals of the CWA. The criteria are based solely on scientific data and judgment concerning ecological and human health effects and must not take into account economic or technological feasibility. The criteria must specify the latest scientific knowledge on:

- All identifiable effects in water on health and welfare, including ecological effects and aesthetics

- Concentration and dispersal of pollutants or by-products through biological, physical and chemical processes

- Effects of pollutants on biological community diversity

The Agency has developed human health criteria for 108 of the 126 priority pollutants and aquatic life criteria for 26 priority pollutants.

3.3.1 Office-Specific Research Needs

The full range of human health effects must be assessed to develop water quality criteria documents and set standards for effluent limitations. Because most of the data used to derive these criteria are based on animal toxicological studies, the Office of Water (OW) needs information that will help resolve the fundamental uncertainties about extrapolating from the experimental animal to the human situation and from the high-dose experimental exposures to the low concentrations generally encountered in the environment. Emphasis is placed on the uptake and pharmacokinetics of the pollutants from the gut after ingestion via water, the skin, or exposure to wastewater sludges.

From a practical perspective, human health data are a low priority for the Office of Water for two reasons:

- Human health criteria have been developed for most of the priority pollutants, while ecological criteria have been developed for only a few

- Health effects criteria for water quality do not have to be based on human data; adverse effects to wild animal species provide a sufficient basis for standard setting

Many animal species live in water; thus, they provide a convenient means of setting the water quality standards and monitoring compliance. The Office of Water does need human data on health effects, however, to compare with those gathered from animal species. Work is needed on structure-activity relationship (SAR) methods and databases for predicting effects in various species; in addition, extrapolation research is needed to link a variety of effects in aquatic species to human disease outcomes.

Methods development for short, simple tests to evaluate the effects of pollutant mixtures, especially in wastewater and sludges, is a major health need for OW. Tests are needed for monitoring the maintenance of water quality and compliance with the standards. Tests and procedures are also needed to couple bioassays with chemical fractionation to identify the toxic components of waste waters and sludges. This information would be used to develop targeted, cost-effective control technologies that remove only those contaminants associated with biologic activity.

3.3.2 Research Plan

The health research program in support of the water quality program is quite small at this time, capable only of providing technical support and guidance, and will be further reduced over the next three to five years. In that time period, work will be limited to identifying the needs for health research in this area. This effort will include background analysis to identify needs for physiologically based pharmacokinetics, SAR approaches, and databases; it may also take the form of workshops to examine the state-of-the-art of nonhuman bioassays for predicting human effects, and vice versa, as a basis for defining future needs and the most appropriate ORD health research program to meet those needs.

3.4 OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

EPA's mandate to protect human health and the environment from pesticides and toxic substances is provided under two acts:

- The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which gives EPA the authority to regulate the distribution and use of pesticides

- The Toxic Substances Control Act (TSCA) of 1976, which gives EPA the authority to prohibit or restrict the manufacture, distribution, use, and disposal of chemicals that present "unreasonable" environmental or human health risks

Federal Insecticide, Fungicide, and Rodenticide Act. Under FIFRA, EPA regulates the distribution and use of pesticides, plant regulators, defoliants, and desiccants (hereafter referred to collectively as pesticides). Pesticide companies are required to generate data on the toxicity and environmental characteristics of the active ingredients in their products, i.e., the ingredients that bring

about the product's intended function. The Agency evaluates these data to determine whether a pesticide should be registered (if it is a new pesticide) or reregistered (if it is an existing pesticide, already registered for use). If new data suggest that an existing product may pose a hazard, EPA performs a special review to determine whether the product should be taken off the market.

New Pesticides. All new pesticides must be registered by EPA before they can be distributed and used. To support a registration application, EPA may require the manufacturer to perform as many as 150 different technical studies and data submissions. The health-related data EPA can request include results of acute studies, subchronic studies, chronic feeding studies, oncogenicity studies, and metabolism studies. EPA can also request data concerning the potential for human exposure. If a product is identical or substantially similar to (e.g., has the same active ingredient as) an existing pesticide, the requirements for new testing data may be reduced.

EPA evaluates the data to determine "any unreasonable risk to man or the environment." At present, there is no guidance or legal precedent to define the level of risk considered acceptable under FIFRA. In practice, for carcinogenic pesticides, a risk of 10^{-4} to 10^{-5} from dietary exposure is a cause for concern, and the Agency considers methods to reduce the risk, such as limiting the product's use or denying registration. If EPA finds that the product does not pose an unreasonable risk, the pesticide is registered for use.

As part of the evaluation of applications, EPA sets a negligible risk tolerance level for food-use pesticides on raw agricultural commodities and a "no-risk" tolerance level on processed food. A tolerance is an amount or concentration of the pesticide residue on food that will not pose an excessive risk. EPA's authority to set tolerances is provided by the Federal Food, Drug, and Cosmetic Act. The act stipulates that tolerances be set at levels deemed necessary to protect the public health, while taking into account the need for "an adequate, wholesome and economical food supply."

Existing Pesticides. The 1988 amendments to FIFRA and the associated regulations require manufacturers to submit more extensive testing data than previously required. To make sure that all pesticides currently in use meet the new FIFRA requirements, previously registered pesticides must be reregistered. Registrants seeking reregistration must compare the data in their current registration with the new requirements and supply any missing data within prescribed deadlines. EPA must review the data submitted to identify any further data gaps and, after the final data submission by industry, must comprehensively examine all data submitted to support pesticide reregistrations. Based on this review, the Agency initiates appropriate regulatory action.

The 1988 amendments substantially increase the number of chemicals to be evaluated by the Office of Pesticide Programs. The reregistration program covers approximately 600 active ingredients in 35,000 pesticides. The entire reregistration process must be completed in 3 to 9 years after enactment of the 1988 amendments (i.e., by about 1991-1997).

Special Review. EPA's special review procedures are initiated after evaluation of data supplied for reregistration, if the data indicate that a pesticide may present an unreasonable risk, including:

- A risk of serious acute injury to humans
- A risk of inducing in humans an oncogenic, heritable genetic, developmental, or reproductive effect, or a chronic or delayed toxic effect
- A risk to humans sufficiently large to merit a determination of whether its benefits exceed its risks
- A risk to nontarget organisms (e.g., acute or chronic toxicity or adverse reproductive effects)

In a Special Review, EPA thoroughly evaluates the risks and benefits of the pesticide, including acute and chronic effects, and the exposure potential for farm workers, bystanders, and consumers. The Agency then decides whether to cancel, suspend, or modify the registration.

The Toxic Substances Control Act. TSCA gives EPA the authority to regulate new and existing chemicals, excluding pesticides, tobacco, tobacco products, nuclear material, firearms, ammunition, food and food additives, drugs, cosmetics, and devices. EPA can restrict manufacture, processing, distribution, commercial use, or disposal if the substance presents "an unreasonable risk of injury to health or the environment." To evaluate chemicals, EPA may require industry to submit extensive testing information on the risks of particular substances. Under TSCA,

EPA evaluates both new chemicals and existing chemicals.

New Chemicals. Under Section 5 of TSCA, businesses must notify EPA at least 90 days before manufacturing or importing a chemical substance for commercial purposes. This notification is called a premanufacturing notification (PMN) and must include the following health information:

- Test data in the submitter's possession or control (often, minimal data are submitted because many submitters have only minimal data in their possession, and EPA cannot require them to generate data for the initial PMN submission)
- Descriptions of health and environmental effects data that they know of or can reasonably ascertain
- Information on chemical identity
- Information on the projected volume manufactured as well as increase in magnitude and duration of exposure and method of disposal

EPA must review the information within 90 days (unless for good cause EPA extends the review period for an additional 90 days). If EPA identifies a potential unreasonable risk that can be evaluated through testing the substance, the Agency can require the submitter to perform further testing of the substance before approving the product for use in commerce. EPA can regulate the substance if it finds that it will present an unreasonable risk to health or the environment. If EPA does not regulate the substance or require testing, manufacture or import can proceed as soon as the review period expires. Businesses must also submit PMNs before manufacturing or processing any chemical substance for a "significant new use" (generally a change in use that may increase human exposure). In making this determination, the Agency must consider all relevant factors, including:

- The projected volume of manufacturing and processing
- The extent to which a use changes the type or form of exposure to human beings or the environment
- The extent to which a use increases the magnitude and duration of exposure to human beings or the environment
- The reasonably anticipated manner and methods of manufacturing, processing, distribution in commerce, and disposal

Existing Chemicals. Section 4 of TSCA gives EPA the authority to require testing of existing chemicals. To implement this authority, EPA must find that:

- The chemical may pose an "unreasonable risk" to human health or the environment; **or** that the chemical is produced in "substantial" quantities, which could result in substantial or significant human exposure or substantial environmental release, **and**
- Insufficient data or knowledge exist about the health or environmental effects of the chemical to reasonably determine or predict the impacts of its manufacture, processing, distribution, use and/or disposal, **and**
- Testing is needed to develop such data

If EPA makes all these findings for a specific chemical or category of chemicals, the Agency may issue a rule requiring industry to test the substance(s). The rule may prescribe the effects to be investigated, the tests to be conducted, and the experimental test guidelines to be used. The TSCA statute itself details many of the studies that may be required. EPA periodically publishes test guidelines and is required by TSCA to review each test standard at least once a year and revise it where warranted.

3.4.1 Office-Specific Research Needs

3.4.1.1 Federal Insecticide, Fungicide, and Rodenticide Act

Hazard Identification for New and Existing Chemicals. Under FIFRA, EPA requires and evaluates test data for new chemicals, but relies on industry to perform the tests. EPA's research needs therefore fall primarily into two areas: 1) development and refinement of methods to be incorporated into test guidelines and protocols, and 2) evaluation of data. In setting test guidelines, EPA must first determine the health endpoints for which the product should be tested. Ideally, EPA should be able to recommend, through guidelines, a battery of tests that will capture all the major health endpoints of concern. EPA then develops reliable, valid tests for these endpoints, and interprets the human health significance of the data.

Test method development and validation are ongoing processes. As new methods are developed, they are validated before they can be used to screen chemicals and/or assess their toxicity. The validity of a test regards what the test measures and how well it performs that task. When validating testing guidelines, researchers assess the test's validity in a number of areas:

- **Content:** Does the test cover a representative sample of the domain to be measured?

- **Construct:** What is the extent to which the test measures a theoretical construct or trait (e.g., intelligence, anxiety)?

- **Criterion:** How effective is the test in predicting an outcome in specific situations (e.g., toxicity)? Two types of criterion-related validity assessments are measurable:

  - Concurrent validity measures: To what extent do other test methods yield similar results to the method in question (e.g., diagnosis of existing disease status)?

  - Predictive validity measures: To what extent do effects noted in one experimental situation predict the effects found in another (e.g., animal-to-human extrapolation issues)?

The Agency has already developed several standard tests that have been incorporated into testing guidelines. Program officials are reasonably satisfied with some of the current protocols; however, ongoing research is needed 1) to refine existing test protocols in areas such as neurotoxicity and reproductive toxicity, and 2) to develop shorter-term, more cost-effective, and more accurate methods capable of assessing a greater variety of noncancer endpoints and multiple endpoints simultaneously so that more information can be obtained from each protocol. A tiered approach to testing has been considered, where the first tier is used to rapidly screen substances for potential toxic effects, and subsequent tiers are used to assess toxicity with greater accuracy and specificity. New methods at both tier levels are needed to assess immunotoxicology, potential carcinogenicity for nongenotoxic agents, reproductive toxicity, endocrine effects, and neurotoxicity.

A testing strategy has been developed to assess the possible toxicity of microbial pesticide control agents (MCPAs) in human populations. Research is needed to determine if MCPAs can survive and interact with rodent and human tissue, to compare routes of exposure, and to investigate observed mortality in mice.

Additional structure-activity research is needed to help define patterns of activity among certain classes of chemicals. This information will be used to help define which tests are appropriate for particular classes of chemicals.

In terms of microbial pesticide control agents (MPCAs), the health research needs of the Office of Pesticide Programs (OPP) are very specific. Issues include the significance of persistance and/or lack of clearance of MPCAs from animals exposed via inhalation; the significance of the mortality observed with *Bacillus thuringiensis* in the current pumonary research; comparison of intraperitoneal exposures to inhalation and intravenous exposures; the significance of baculoviruses in vertebrate cells; and determining whether a significant interaction exists between viral pesticides and vertebrate viral pathogens. These issues are of high priority to the Office of Pesticide Programs.

As part of the reregistration process, EPA must specify tests and evaluate data for existing pesticides. The research needs described here for new pesticides are therefore also valuable in supporting the reregistration activities of the Office of Pesticide Programs. The large number of chemicals that must be evaluated for reregistration will place a greater burden on EPA's resources to evaluate chemical data. Thus, the need for research to develop rapid and reliable tests for chemical toxicity will likely increase in the future.

Dose-Response Assessment for New and Existing Chemicals. In the pesticides program, the potential human health effects of pesticides generally must be evaluated based on data derived from high-dose exposures in whole animals or *in vitro* tests. Therefore, dose-response issues are a high priority for the pesticides program, particularly for the numerous evaluations that will be done under the reregistration program set up under the 1988 amendments. Research on mechanisms/sites of action and pharmacokinetics is needed in the many areas relevant to interpreting and evaluating data, including:

- Fate and disposition of pesticides, especially as related to dermal absorption and route-to-route extrapolation

- Extrapolation of effects observed at high doses to effects observed at low doses
- Extrapolation of animal data to humans
- Extrapolation from acute to chronic exposure and from one route of exposure to another

In some cases, observed health effects are temporary or transient; the body can adapt to the insult or repair the damage when exposure ceases. Thus, research is needed to study injury and repair mechanisms following chronic and acute exposure. These data would help the tolerance-setting process and the ability to interpret the significance of toxicological effects.

As noted above, EPA must specify tests and evaluate data for existing pesticides as part of the reregistration process. The research needs described for dose-response for new pesticides are therefore also valuable in supporting these reregistration activities. The large number of chemicals that must be evaluated for reregistration will place a greater burden on EPA's resources to evaluate chemical data. Thus, the need for research to perform research to support accurate data interpretation, assessment, and extrapolation will likely increase in the future.

Exposure Assessment for Existing Pesticides. Biomarkers"measures of samples of human tissues or fluids (e.g., blood lead levels)"provide information about human exposure, response, and/or susceptibility to future challenge. Biomarkers come directly from humans and offer great promise for 1) evaluating exposure and response in populations, 2) evaluating the effectiveness of control technologies, and 3) enabling early intervention in the cascade of events from exposure to effect to prevent disease. When used in experimental animals and when coupled with pharmacokinetic experimental and modeling studies, they will provide a means to extrapolate between doses and species, and to understand mechanisms of disease.

Biomarker research is important across the full array of adverse outcomes. Research is particularly needed to develop markers for exposure, reproductive function, genotoxicity, cancer, and pulmonary effects, and for outcomes associated with specific classes of pesticides. Such biomarkers could be used to evaluate whether humans respond to various levels of pesticide exposures. This information would provide a basis for refining tolerance levels and evaluating worker safety.

Exposure research is needed to better assess the level of exposure that is occurring from pesticides currently in use, i.e., via consumption of contaminated drinking water, inhalation of emissions from incineration of pesticides, food consumption, and exposure through skin contact during application and use. This research should focus on improving comprehensiveness, developing better surrogates (including biomarkers), and improving validation. Research is also needed to develop methods for monitoring exposure to MCPAs.

3.4.1.2 The Toxic Substances Control Act

Hazard Identification for New and Existing Chemicals. Most PMN submissions contain little or no substantial toxicological data, and EPA is not given much time to review the data. For these reasons, the program office relies heavily on an analysis of a chemical's structure to predict its activity. Substantial research, including data base development, is needed to refine and quantify structure-activity relationships for use in predicting the toxic effects of untested chemicals.

Currently, most testing guidelines are limited to conventional toxicity endpoints. Test method development is a major research need for the TSCA program. Validated methods are needed for all important toxicologic endpoints, including particularly neurotoxicology, developmental/reproductive toxicology, genetic toxicity, and immunotoxicology endpoints. Also, short-term tests (e.g., *in vitro* tests) are needed for screening for effects in all target systems. Short-term screening methods would enable OPP to develop abbreviated protocols that would help reduce the economic burden on industry (so as not to provide a disincentive to manufacture or import). Several *in vitro* tests look promising, based on early validation efforts with a limited number of chemicals. Research is needed to evaluate them using more stringent criteria. Interlaboratory validation is also needed.

In support of PMN registration evaluation, tests are needed to evaluate the potential pathogenicity and toxin production in a variety of GEMs. Eventually, rapid short-term assays will be needed to replace the more expensive long-term assays now under development.

A specific research area is the potential health impacts of conventional microorganisms and GEMs released to the environment for commercial purposes. Such research is needed to better understand microbial characteristics responsible for infection, pathogenicity, and genetic exchange. EPA will use Agency-sponsored biotechnology

ecology studies, approved environmental releases, industrial and other federal agency data bases to generate, evaluate, and validate appropriate test methods and risk parameters.

For existing chemicals, EPA must determine which chemicals should be tested, develop test methods, and provide scientific support for any regulatory action. Screening, test method development, and data evaluation are therefore important ongoing research needs for the program office. Such research helps increase the efficiency and acccuracy with which the program office can assess chemicals and make regulatory decisions. In particular, rapid, inexpensive *in vitro* assays are needed for screening and for enhancing our understanding of mechanisms of action.

Several compounds of interest to the program office, such as dioxin and perchloroform, appear to be nongenotoxic carcinogens (i.e., they cause cancer by some method other than direct interaction with DNA). These compounds cannot, therefore, be evaluated using the standard risk assessment process for carcinogens. Research is needed to elucidate the mechanism of action of nongenotoxic carcinogens to facilitate risk assessment, particularly animal-to-human extrapolation.

Dose-Response Assessment for New and Existing Chemicals. Dose-response research is a high priority for the TSCA program. An understanding of mechanisms of action would aid in developing short-term tests for certain endpoints, improve the evaluation of risk to humans, provide a cost-effective means of screening large numbers of chemicals, and also prove useful in understanding why different species react differently to the same chemical. Such understanding would increase the accuracy of interspecies extrapolation in chemical assessments. In particular, mechanistic studies are needed for noncancer effects and nongenotoxic carcinogens. Research is also needed to develop and improve quantitative biomathematical modeling of dose-response data from noncancer endpoints.

Exposure Assessment for Existing Chemicals. Research is needed to develop biomarkers of exposure that would provide important tools for more accurately assessing the extent and degree of exposure in human populations. Such information would help OPP determine whether to require testing under Section 4 of TSCA. Extensive pharmacokinetic research is needed to validate biomarkers of exposure and, where feasible, to determine whether they could also be used as biomarkers of effect. Biomarkers of effect would also provide information valuable for extrapolating animal test data to human outcomes. In particular, biomarkers that are predictive of neurotoxic endpoints and carcinogenesis should be developed.

3.4.2 Research Plan

The Health Effects Research Laboratory has developed a research plan to address the critical needs of the program office for pesticides and toxic substances. The research focuses on two primary outputs: test methods development/validation and data interpretation. The method selected for development may be pesticide- or toxic-substance-specific or may be more generic to cover classes of chemicals. The research process involves method selection, method development, refinement or evaluation of the methodology, validation, guideline development, and data interpretation. The research falls under the broader categories of hazard identification and dose-response assessment described below.

3.4.2.1 Pesticides

Hazard Identification. To address the ongoing need for improved testing methods, HERL will conduct research to investigate the full array of potential adverse effects from exposure to pesticides, including"but not limited to"neurotoxic, reproductive, and immunotoxic effects. Basic research will involve refining existing techniques and creating new ones in these scientific disciplines. Applied research efforts will involve evaluating and interpreting industrial data (both review of raw data and laboratory research to verify and clarify data reported from industry).

- **Neurotoxicity.** Many pesticides work by interfering with nervous system function. Therefore, tests for neurotoxic effects are particularly important in evaluating the potential human health effects of pesticides. Research will continue to develop and validate tests capable of detecting effects at all levels of the nervous system, including behavioral, neurophysiological, cellular, and molecular levels. Since neurotoxicology is a rapidly evolving field, HERL will periodically review and, where appropriate, refine existing tests for potential refinements to make them simpler, more rapid, and more accurate.

  The current first tier test battery for neurotoxicity focuses on detecting the ability of a chemical to cause overt motor, sensory, or autonomic deficits. Research

will be conducted to support new test guidelines for evoked potentials, glial fibrillary acid protein (GFAP) (a neurochemical marker of neural injury), and effects on learning and memory. HERL will also investigate a test for sensory evoked potential, currently being used by some industrial laboratories, for potential use under FIFRA.

Research will be initiated to evaluate the use of tissue cultures for studying potential neurotoxicants *in vitro*. Initially, the approach will be systematically validated by evaluating a number of neurotoxicants known to produce structural deficits *in vivo* and comparing those effects to neuroactive or short-acting psychoactive agents known not to be neurotoxic *in vivo*. Subsequently, chemicals with unknown neurotoxic potential will be compared using *in vivo* and *in vitro* procedures. The overall goal of this research will be to arrive at a validated *in vitro* tissue culture procedure that can be used to assess chemicals for potential neurotoxicity.

Neurotoxicologic research will continue to work within a tiered approach to neurotoxic testing. A first tier battery of tests is currently being validated. Further studies will be conducted to refine the battery and determine the conditions under which it should be used. Eventually, based on the results of mechanistic research described below, tests for effects at the cellular and molecular level may be added to the battery. Another future research area is the development and validation of the second tier of tests, which would be used to confirm the results of the first tier, determine if the effect(s) is(are) primary or secondary, and develop further information about the chemical's toxicity, such as the lowest-observed-adverse-effect level. This information will be important for reducing uncertainty in the risk assessment process.

- **Reproductive toxicity.** Currently, reproductive toxicity relies on fertility as a measure of reproductive damage; however, this endpoint is relatively insensitive under certain circumstances. Also, present tests generally do not provide information on the affected sex, the likely target tissue, or the mechanism of toxicity. Thus, a major need exists for improved testing protocols, as several Agency-sponsored workshops have concluded. HERL will continue research to develop alternative reproductive test (ART) strategies to replace or supplement the current multigeneration tests. This research will develop improved methods to assess testicular, ovarian, and uterine function (e.g, semen evaluations, oocyte toxicity). The research is designed to develop an ART protocol that would detect developmental effects beginning with the period of sexual maturation, and proceeding through the entire reproductive life cycle. A long-term goal of this research is to effectively characterize the affected sex and target site, and information on the homologous nature of the response.

- **Immunotoxicity.** Immunotoxicity testing under FIFRA is currently limited to Subpart M pesticides (i.e., biopesticides). However, immunotoxicity testing guidelines for other pesticides, as well as toxics, is being discussed. Improved testing methodologies are being developed, particularly in species other than the mouse. Also planned is research to improve our capability to predict risk of increased disease based on effects in immune function tests. Research will continue to develop a tiered approach to immunotoxicity testing in several species with particular emphasis on species comparisons and relationship between effects in tier one and susceptibility to infectious, neoplastic or allergic disease in tier 2. This research will include development of a variety of host resistance models in the rat and mouse for use in tier 2 and for species-to-species extrapolation. Research will also be conducted to assess the potential of various types of compounds to produce allergenic and autoimmune effects. This work would be particularly applicable to microbial pesticides, which contain proteins and are generally more likely to be allergenic than chemical compounds.

- **Microbial pesticide control agents.** To better understand the likelihood of opportunistic infection and pathogenicity, researchers will explore the capabilities of MPCAs to invade tissues and interact with vertebrate cells and vertebrate viral pathogens. Research will also be conducted to compare routes of exposure and observed mortality in mice. Also of import is the persistence or lack of clearance of the MPCAs from the lung.

Dose-Response Assessment. Research will be conducted to develop a better understanding of the mechanisms underlying the effects associated with pesticides for which tests are available. Such research will help elucidate the cellular/molecular events that are associated with changes in physiology and function. This research will also enhance understanding of the predictive value of the tests, and the ability to extrapolate data from one species to another. Also, such research may ultimately lead to the development of cellular/molecular tests that could replace or supplement existing protocols. These ef-

forts need to be ultimately linked to pharmacokinetic models such that a complete description of exposure, dose, and outcome can be used in the risk assessment process.

- **Neurotoxicity.** Many pesticides work by inhibiting cholinesterase, an enzyme essential for neuromuscular activity. At low levels, cholinesterase inhibition appears to have little or no effect. At high levels, it can be fatal. Research will include a major initiative to study cholinesterase inhibition. Issues to be addressed include the relationship between cholinesterase activity and other effects observed following pesticide exposure; assessing whether cholinesterase inhibition is an adverse effect, a marker of adverse effect, or a marker of exposure; determining whether the age of exposure affects the vulnerability of the organism to the consequences of cholinesterase inhibition; pinpointing the consequences of long-term exposure to cholinesterase inhibition; and evaluating the developmental effects of cholinesterase inhibition. Clinical and animal studies will be conducted to identify what learning and memory endpoints can be quantified in both rats and humans. Ultimately, HERL hopes to be able to use rats to model the potential learning deficits a chemical may cause in humans. To facilitate animal-to-human extrapolation, studies will be done to correlate electrophysiological and cognitive measures in test species and humans.

- **Reproductive toxicity.** Tremendous gaps exist in our knowledge of how human developmental toxicants behave in animal models, yet many of these toxicants have defined pharmacokinetic and phamacodynamic profiles that could readily be compared across species. One project being developed is a "retrospective risk assessment" to evaluate how a well-developed animal database predicts known human risk. Research will also be performed to develop capabilities for quantitative dose-response modeling of developmental effects, with the ultimate goal of developing biologically based dose-response models. These models will be largely statistically based in the beginning, but will gradually incorporate more biology, such as pharmacokinetic considerations, mechanisms of toxicity, and the presence or absence of thresholds. A critical component will be the establishment of confidence limits about predictive low-dose effects. Such models will be developed first for developmental toxicity and then for reproductive toxicity.

Studies of developmental toxicity will examine the significance of morphological and skeletal variants, such as supernumerary ribs, that are commonly observed in developmental studies. Issues to be addressed include determining whether the effects are adverse and whether related effects can be noted, studying the mechanisms for these effects at the molecular and cellular level, and assessing whether similar effects occur in other species. Approaches pioneered in examining variations in kidney development will be used as a model for these efforts. The role of maternal stress in embryonic development will also be examined. This research will help establish alternative methods for setting the maximum tolerated dose use in standard developmental assays.

The Office of Pesticide Programs has expressed concern about the acute and chronic effects of pesticide exposures on people who apply pesticides. In response to this concern, researchers will examine the reproductive outcomes associated with acute and chronic exposures. HERL will investigate whether these outcomes are adverse and/or reversible, and what the tolerance is to exposure. Research will focus on male reproductive effects (i.e., semen parameters) as well as events occurring during normal estrous cycling and early pregnancy. These mechanistic studies, conducted at the cellular level, will help determine appropriate parameters for evaluation as a function of putative mechanisms that are triggered under differing exposure conditions. In addition, because recent human data suggest that approximately one-third to one-half of all pregnancies are lost in the first two weeks after fertilization, HERL will examine the correlation between ovarian and uterine function in animal models and humans in an effort to develop an animal model for pre-implantation effects. This research will provide the basis for recommendations on identifying hazards to early pregnancy. HERL will also conduct research to study the sensitivity of the nonpregnant female to disruptions in reproductive capabilities.

Researchers in reproductive toxicology will explore the development of different biomarkers. They will examine endocrine parameters and semen quality as a potential biomarkers of effect. Studies will investigate the relationship of sperm alterations to prediction of altered fertility in human and test species. Investigators will also develop biomarkers of genetic change in human and animal cells and develop methods for using DNA adducts as biomarkers of exposure and/or effect. In particular, HERL will ex-

amine the relevancy of DNA adducts for cancer initiation. Research efforts will investigate the significance of gap junctions (e.g., communicator regions between cells) as potential markers for promotion and carcinogenesis. Other work will develop biomarkers of allergenic potential. In particular, this research will investigate the relationship between serum antibody levels and pulmonary hypersensitivity. HERL scientists will also develop biomarkers of increased susceptibility to infectious disease using several immune function endpoints. Biomarkers research will also be conducted to investigate sensitivities of susceptible populations such as field workers, children, and pregnant women.

3.4.2.2 Toxic Substances

Hazard Identification. Research needs for toxic substances are similar in nature to those for pesticides. To address the ongoing need for improved testing methods, HERL will conduct research to investigate the full array of potential adverse effects from exposure to pesticides, including"but not limited to"neurotoxic, reproductive, and immunotoxic effects. Basic research will involve refining existing techniques and creating new ones in these scientific disciplines. Applied research efforts will involve evaluating and interpreting industrial data (both review of raw data and laboratory research to verify and clarify data reported from industry).

- **Structure-activity relationships.** Structure-activity research will address the need for more effective methods to screen PMN chemicals. HERL is developing a molecular similarity index and specialized databases for use in SAR analysis that profile the genetic and developmental activity of chemicals in relation to their structures. HERL will also conduct research to provide a quantitative basis for classifying and prioritizing chemical structures according to their activity. Causal molecular models will be developed that predict the distribution, transformation, deposition, or biological activity of classes of chemicals from computationally available molecular properties. Additional SAR research will explore mechanisms of action of nongenotoxic carcinogens, and developmental and reproductive toxicants, and examine correlations between mechanism and activity. Mechanisms for mutation in germ cells will also be examined in an effort to correlate structure and activity.

- **Developmental toxicity.** In anticipation of future needs, HERL will initiate development and validation of *in vitro* screening assays for detecting developmental hazard. A battery of assays is likely to be required, with guidance provided as to which assays are appropriate for which chemical classes. Similar assays may be established for endpoints of reproductive toxicity, particularly as they relate to the events encompassing spermatogenesis.

- **Genetic toxicology.** HERL will continue the development of test guidelines for evaluating chemically induced heritable damage in the germ line, as well as examine the types of genetic damage that can be caused in diverse organisms.

- **Immunotoxicity.** Researchers will continue the development of test guideline methods for immunotoxicity and develop immunotoxic endpoints as biomarkers of susceptibility to infectious or allergic diseases.

- **Genetically engineered microorganisms.** To address the research need for methods to evaluate GEMs, research will be conducted to investigate the health implications of exposure to GEMs. This will include research on 1) the survival of GEMs in the intestines of rodents and humans; 2) the ability of GEMs to invade other tissues and induce adverse health effects; and 3) the genotoxic and metabolic changes that occur following exposure to GEMs.

Dose-Response Assessment. Dose-response research needs for toxics are similar to those for pesticides. Research will be conducted to develop a better understanding of the mechanisms underlying the effects associated with toxics for which tests are available. Such research will help elucidate the cellular/molecular events that are associated with changes in physiology and function. This research will also enhance understanding of the predictive value of the tests, and the ability to extrapolate data from one species to another. Also, such research may ultimately lead to the development of cellular/molecular tests that could replace or supplement existing protocols. These efforts need to be ultimately linked to pharmacokinetic models such that a complete description of exposure, dose, and outcome can be used in the risk assessment process.

- **Genetic toxicology.** The structure-function properties of biological receptors will be studied with the goal of developing molecular-level models of the mechanisms of toxicity. Research will include extrapolation studies to establish the experimental basis for relating molecular dose to DNA adducts to in-

duced genetic effects, and to determine whether these relationships established using laboratory animals can be extrapolated to humans. Also, research will be conducted to examine the validity of extrapolating carcinogenic effects from rodents to humans, and to establish the shape of dose-response curves. This research will support the development of biologically based dose-response models for carcinogens. Mechanistic research will be conducted to determine the role of environmental chemicals in the expression of oncogenes and tumor suppressor genes. Such research will establish a biological basis for developing biomarkers of preneoplastic and neoplastic lesions in exposed or susceptible human populations.

The use of DNA adducts or cytogenetic effects as measures of internal or target dose will be explored. This research is designed to improve the accuracy of dose determinations for interspecies and route-to-route extrapolation. Basic research will be conducted to understand the fundamental interspecies differences between the response of rodent and human cells to genotoxic agents. Research has already indicated that human cells may be more capable than rodent cells of repairing at least some DNA lesions, suggesting that human cells may be less sensitive to genotoxic agents. Molecular techniques will be used to define and quantify the specific interspecies differences.

To address the need to understand nongenotoxic carcinogenesis, HERL will conduct research to identify nongenotoxic carcinogens and their mechanisms of action, and to develop extrapolation models for this class of carcinogens. Areas to be highlighted are gap junction intercellular communication, alterations in gene expression, and indirect genotoxic carcinogenesis (e.g., peroxisome proliferators, free radical generators).

- **Developmental toxicity.** Currently, developmental research focuses on fetuses exposed *in utero* because there are few acceptable procedures for evaluating effects of postnatal exposure. However, OPP is particularly interested in research addressing the potential developmental effects in pups exposed via lactation from mothers who were exposed either dermally or via inhalation. Exposing females by these routes requires removing them from their pups at frequent and prolonged intervals during the lactation period. This maternal deprivation itself causes stress to the pups that may confound study results. HERL will examine the role of maternal stress on the growth, physiology, biochemistry, and behavior of pups. Results of this research will supplement guidelines for developmental neurotoxicity testing. Research will also be conducted with the goal of developing procedures for pharmacokinetic and biologically based dose-response assessments for reproductive and developmental toxicity, as described earlier.

- **Neurotoxicology.** In support of toxic substances dose-response research needs, HERL will conduct research to better understand the human neurological significance of exposure to toxic substances. The research will focus on development of an animal model of personality changes, learning and memory deficits seen in humans exposed repeatedly to pesticides; the mechanism of compensation or tolerance after repeated exposure; and interactions of toxicants with central nervous system depressants (tranquilizers).

- **Biomarkers.** To meet the research need for biomarkers of effect and exposure, research will be conducted as described above (see Exposure Assessment under Pesticides). Also gene mapping techniques will be used to study genetic alterations in human and rodent precancerous and cancerous lesions as potential biomarkers of effect and/or exposure.

3.5 OFFICE OF EMERGENCY AND REMEDIAL RESPONSE

Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980, and subsequent reauthorization and amendments (Superfund Amendments and Reauthorization Act [SARA] of 1986), EPA is responsible for identifying sites from which releases of hazardous substances might occur or have occurred. With only a few exceptions, Superfund coverage extends to all sources of releases and all means of entry of a substance into the environment. Once a site is identified, the Agency must ensure that the site is cleaned up, either by removal of the wastes or remediation.

The procedures through which Superfund is implemented are outlined in the National Oil and Hazardous Substances Pollution Contingency Plan (NCP). After the Agency is made aware of the existence of a site, it performs a site evaluation. Based upon that evaluation, the site is slated either for a short-term removal action or longer-term remedial action. Within predetermined intervals after completion of a remedial action, EPA must

evaluate the continued effectiveness of the action through a post-closure review.

The OHR/HERL research program relating to Superfund is structured to address the special health research needs of the site evaluation, removal, remedial, and post-closure review programs. At an early stage in the Superfund process, the Agency for Toxic Substances and Disease Registry (ATSDR) performs a health assessment for each site. Based on this assessment, ATSDR may perform an epidemiological study. ATSDR's decision about whether and when to perform an epidemiological study is relatively independent of the EPA cleanup process. Because EPA and ATSDR interact, both formally and informally, during these processes, however, OHR/HERL research program results may provide direct input to ATSDR epidemiology studies. The OHR/HERL program in this area is new; thus, details of program implementation have not yet been fully determined.

Site Evaluation Program. This program, sometimes called the preremedial program, supports both the removal and remedial programs by conducting preliminary assessment (PA) and site inspection (SI) activities. Removal PAs are based on readily available information, which may include the preliminary ATSDR health assessment, if available. Removal SIs are performed if more information is needed, and if the National Contingency Plan does not specify collection of any particular data for these activities. No risk assessment is performed, although some estimate of the magnitude of the threat is made.

Remedial PAs are similar to Removal PAs. If a Remedial SI is performed, the lead agency collects additional data necessary to apply the Hazard Ranking System (HRS); even more data are collected if necessary to better characterize the release and facilitate the effective and rapid initiation of the remedial investigation/feasibility study (RI/FS; see Section 3.5.1.3). These activities fall primarily under hi. In general, they are based on comparing what is discovered about conditions at a specific site against what is already known about contaminants and environmental levels of concern for those contaminants (in the form of RfDs, cancer potency factors, and LD_{50}). When, as often happens, sufficient data are not available for the specific contaminants, evaluations may rely upon structure-activity relationships.

Removal Program. The removal program, which is limited (with a few exceptions) by statute to one year and $2 million, is geared toward mitigating, abating, minimizing, stabilizing, or eliminating a release or threat of a release that may threaten public health, welfare, or the environment. Among the criteria used to determine whether a removal action is necessary are:

- Actual or potential exposure of human populations, animals, or the food chain to hazardous substances, pollutants, or contaminants
- High levels of hazardous substances, pollutants, or contaminants in soils largely at or near the surface that may migrate (proposed section 300.415 of NCP)

This information is obtained from site evaluations and knowledge of the current site conditions.

An important concern in managing a removal operation is community relations. A designated spokesperson is assigned to inform the community of actions taken, respond to inquiries, and provide information concerning the release. EPA must have accurate and appropriate information to provide to the community on the potential risks associated with any site and on how the data should be interpreted. At a minimum, EPA should be able to communicate 1) information about exposure and the relationship between ambient and internal exposure; and 2) information about the relationship between magnitude of exposure and health effects (dose-response issues).

Based on the hazard assessment and other considerations, ATSDR may consider conducting one of several types of human health (e.g., epidemiology) studies at a site at which a removal action is scheduled. A primary criterion in ATSDR's decision to perform either an exposure study or an epidemiological study is the availability of an adequate test or biomarker of exposure: the ability to incorporate biomarkers of exposure into a study allows much more accurate assignment of the study population to groups.

Remedial Program. This program implements long-term solutions (i.e., reduces controls, or eliminates risks to human health and the environment) to problems posed by a site. A central part of this program is the RI/FS, which assesses the potential risk to human health posed by the site if no action is taken (baseline risk assessment), as well as the relative risks likely to result following various cleanup options.

Factors considered in assessing risk at a site include:

- The nature (e.g., toxicity, bioaccumulation potential), medium, and concentration of substances
- The location of nearby sensitive populations

When systemic toxicants are present, cleanup goals are set so that even sensitive subgroups of the human population could be exposed to the remaining toxicants for a lifetime without appreciable risk of significant adverse effect. When known or suspected carcinogens are present, cleanup goals are set to ensure that concentration levels represent an excess upper-bound lifetime cancer risk to an individual of between 10^{-4} and 10^{-7}, using information on dose/response relationships.

Several features of the risk assessments performed during the RI/FS make them atypical of other risk assessments performed in the Agency. First, the hazard identification stage of the risk assessments may have to address the potential toxicity of chemicals for which little data are available. Thus, knowledge of SARs is particularly helpful. Second, if bioremediation is being considered as a cleanup option, the feasibility study may need to evaluate the potential hazard posed by the microorganisms (or substances they produce) employed in the process. These two concerns are hazard identification research issues.

In terms of dose-response relationships, risk assessments performed as part of the RI/FS must consider the pollutant mixtures found at most sites rather than individual pollutants. Assumptions must be made about how to evaluate risk associated with exposure to mixtures. Second, Superfund risk assessments must consider multiple pathways/routes of exposure. For this reason, knowledge about route-to-route extrapolation and dosimetry are of particular importance.

As with the removal program, the remedial program requires a community relations plan that handles public information needs appropriately. Again, EPA must have accurate and appropriate information to provide to the community on the potential risks associated with the site and on the appropriate interpretation of the data available. At a minimum, EPA should be able to communicate information concerning 1) exposure and the relationship between ambient and internal exposure, and 2) the relationship between magnitude of exposure and health effects.

In remedial as in removal situations, ATSDR may consider conducting epidemiological studies. Again, the availability of biomarkers of exposure is critical to that decision: without them, the study population cannot be accurately assigned into groups.

Post-Closure Program. The CERCLA amendments require reviews at least every five years at sites where remedial actions leave hazardous substances, pollutants, or contaminants above levels that allow for unrestricted use and unlimited exposure for humans. If a selected remedy is no longer considered protective (e.g., standards have changed), EPA will evaluate and take additional action at affected sites to mitigate the threat. No procedures have yet been promulgated to accomplish these post-closure reviews, and the development of such procedures is an emerging issue for the program.

In many respects, post-closure activities may be viewed as pollutant-monitoring activities. Biomonitoring tools are some of the most promising for ensuring safety at remediated sites, at least until further advances in the following areas:

- Predictions of pollutant transformation in the environment
- Monitoring of all likely pollutants
- Predictions of the health impact of exposure to various mixtures of pollutants

These biomonitors would serve as sentinels for hazard identification at the site.

3.5.1 Office-Specific Research Needs

3.5.1.1 Site Evaluation Program

Health research data are needed to increase the number of chemicals for which hazard potential may be accurately identified.

3.5.1.2 Removal Program

Dose-Response and Exposure Assessment. Because of the typically time-critical nature of its work, the removal program relies on generally available information. Health research is needed to provide background

data that the Agency can use to interpret for the public the relationship between:

- Ambient and internal exposure
- Internal exposure and risk of adverse health effects

In addition, health research is needed to provide biomarkers of exposure and effect so that epidemiologic studies can be performed when appropriate.

3.5.1.3 Remediation Program

Dose-Response and Exposure Assessment. Health research is needed to improve:

- The baseline risk assessments that are part of the RI/FS
- The risk assessments included in the evaluation of cleanup options
- Availability and interpretability of biomarkers of exposure and effect

3.5.1.4 Post-Closure Program

Research needs in this area include the development/validation of test systems for *in situ* biomonitoring.

3.5.2 Research Plan

3.5.2.1 Site Evaluation Program

Work in this area falls under the category of hazard identification. HERL research efforts in site evaluation are focused on improving the database used to identify the hazard potential of constituents. In support of that goal, research will concentrate on improving the use of SAR for identifying hazard; this strategy was chosen because it is the most efficient means to the desired end when data are unavailable and testing cannot be mandated. In the short term, efforts will be directed to genetic toxicity as an endpoint, because of the substantial database available from which to develop SAR theories and models. As more data become available, the SAR approach will be extended to other forms of toxicity, including developmental toxicity and neurotoxicity.

3.5.2.2 Removal Program

Dose-Response and Exposure Assessment. Research in this area is focused on these goals: 1) providing information to the Agency that can be used in the community relations program to explain and interpret the significance of site-specific data, and 2) providing information about biomarkers for incorporation into site-specific human health studies. To achieve these goals, work will concentrate on biomarkers, particularly in terms of exposure and dose-response assessment. This strategy was chosen because biomarker research will foster a better understanding of the relationship between ambient and internal exposure (i.e., dose to the target tissue), and between internal exposure and effect. This information will be useful in the community relations program.

Biomarkers of exposure could be used to evaluate exposure to any of the hundreds of chemicals of concern at Superfund sites. ATSDR health assessments (from the 288 NPL sites evaluated since enactment of SARA) indicate that the most commonly found classes of substances are:

- Volatile organic compounds (VOCs) (64 percent of sites)
- Metals (47 percent of sites)
- Polycyclic aromatic hydrocarbons (PAHs) (27 percent of sites)
- Polychlorinated biphenyls (PCBs) (25 percent of sites)

HERL/OHR work will focus on substances 1) that are most likely to be found at Superfund sites, and 2) for which valid biomarkers of exposure are currently not available. Identification of valid exposure biomarkers, coupled with knowledge of their kinetics in tissues, cells, and fluids, will not only facilitate the documentation of exposure, but will allow researchers to reconstruct exposure history and make quantitative estimates of exposure.

Removal rather than remediation is likely to occur at sites where exposures could be relatively high for a relatively short period of time. At these sites, public concern may be high. Human health studies, if initiated, would benefit from objective measures of toxic effect; these could be made in humans and could be related to indices of exposure. While many endpoints are important (e.g.,

neurotoxicity, reproductive toxicity), research in this area will focus on those that are 1) of greatest concern from a short-term exposure perspective (since in the removal program exposures are likely to be relatively short in duration), and 2) most likely to reveal significant gains with limited resources and limited time.

The field that appears to best fill these criteria is reproductive toxicology. Concern about reproductive dysfunction is high, problems in this area are likely to be manifest after reasonably short exposures, and test methods are available for evaluating male reproductive function in both humans and laboratory animals. Significant research needs in this area include:

- Validation of computer-assisted analysis of sperm as a method for quantitative assessment of sperm motility and morphology
- Evaluation of the sensitivity of sperm analysis to toxicant-induced disruption
- Development of a better understanding of the relationship between alterations in sperm and reproductive dysfunction

In addition to serving as a biomarker of reproductive effect, sperm may provide a host for chemical adducts, which could be used as biomarkers of male reproductive system exposure to environmental chemicals. Future work may focus on development of sperm adducts as biomarkers of dose to a target tissue.

3.5.2.3 Remediation Program

Research in this area is focused on improving the risk assessments performed during the RI/FS, and on improving the availability and interpretability of biomarkers of exposure and effect. Work will concentrate on those aspects of risk assessment that are relatively unique to the Superfund process, and on biomarker research that has a particularly high probability of being useful in human health studies at Superfund sites. Through this strategy, research efforts will be directed to three risk assessment activities: hazard identification, exposure assessment, and dose-response assessment.

Hazard Identification. Work in this area will focus on developing SAR.

Dose-Response and Exposure Assessment. When ambient concentrations are high enough that humans have been or are being exposed, biomarkers of exposure can be used to document that exposure. Most useful would be biomarkers that allow exposure to pollutant mixtures to be evaluated; some work will be done in this area, particularly with protein adducts as the biomarkers under study. The first protein adducts to be examined will be those produced by exposure to PAHs; this class of compounds is often found at Superfund sites, and the data available suggest that this research may be fruitful. Subsequent efforts will address exposure biomarkers for other classes of compounds.

Three research issues will be explored in dose-response assessment: route-to-route extrapolation, biomarkers (for target dose), and pollutant mixtures.

- **Route-to-route extrapolation:** Often data are only available for one route of exposure, but plausible exposure scenarios suggest multiple pathways and/or routes. In the short term, research will focus on dosimetry via the inhalation route of exposure. In the longer term, research will focus on the relationship between oral and inhalation exposure. Since dosimetry is age-dependent, near-term research will address the principles underlying this age-dependence (e.g., geometry of the airways).

- **Biomarkers:** Just because exposure has occurred does not necessarily mean that the chemical(s) in question reached its target site(s). Understanding the relationship between exposure and dose to the target site can reduce significantly the uncertainty in Superfund risk assessments. As part of the HERL biomarkers strategy, research efforts in this area will focus on already-developed biomarkers of target dose (i.e., DNA adducts) to determine 1) their life in tissues, 2) relationship to exposure, 3) levels in human tissue, and 4) relationship to genetic effects. To the extent possible, this work will proceed using *in vitro*, laboratory animal, and human studies.

- **Pollutant mixtures:** Most Superfund sites contain mixtures of pollutants. As a result, risk assessments performed during the RI/FS are chemical mixture risk assessments, and research needs in this area emphasize the importance of mixtures. The Superfund pollutant mixtures research strategy represents an integral part of the HERL mixtures research strategy. To meet Superfund needs, HERL will perform research directed at understanding the validity of the

current assumption that the effects of components in a mixture are additive.

Much of the pollutant mixtures work to date has focused on short-term *in vitro* tests of specific genetic endpoints, mostly following exposure to combustion emissions. In the long term, this scope will be broadened through an emphasis on *in vivo* exposures and other (e.g., systemic) endpoints. In addition, research will focus on mixtures likely to be found at Superfund sites, and on the comparative potency of residual toxicity following various cleanup options.

3.5.2.4 Post-Closure Program

Work in this area falls under hazard identification. Present methods for *in situ* biomonitoring focus on 1) genetic endpoints, and 2) plant-based systems. In the near term this focus will continue. Plant-based test systems must be validated against more conventional test systems. In addition, field studies must be validated against laboratory studies that use 1) pure chemicals, and 2) field samples. In the long term, efforts will turn toward ensuring that biomonitoring tools developed for application during post-closure review are protective against other forms of toxicity as well as genotoxicity. This new goal will almost certainly necessitate exploration of animal-based test systems.

3.6 OFFICE OF SOLID WASTE

Under the Resource Conservation and Recovery Act (RCRA) and its amendments, EPA manages a comprehensive program to address the national problem of solid waste. The program is designed to:

- Protect human health and the environment from the potential hazards of mismanaged waste
- Conserve energy and natural resources
- Reduce the amount of waste generated
- Ensure that wastes are managed in an environmentally sound manner

RCRA applies to all solid waste, which is broadly defined as "garbage, refuse, or sludge or any other waste material," including liquids, solids, semisolids, or contained gases.

The criteria applied for managing a particular waste depend on its characteristics. To accomplish the mandate specified by RCRA and subsequent amendments, the Office of Solid Waste (OSW) must first characterize wastes. This characterization then determines which program covers the management of that waste"either Subtitle C (hazardous waste) or Subtitle D (nonhazardous waste). OSW programs are designed to track, permit, and enforce requirements for waste management. OSW cannot request health research from the regulated community, although it may require industry to test the "characteristics" of the waste. The OHR/HERL research program is structured to fill that gap by addressing the special health research needs associated with waste characterization, hazardous waste, and nonhazardous waste. Because the research program is new, details of program implementation have not yet been fully determined.

Waste Characterization. Waste characterization is a pivotal feature of the hazardous and solid waste program: the results of this activity determine the regulatory path taken by the waste. Wastes are defined as hazardous on the basis of either:

- Their "characteristics"
- Federal listing (in Appendix VIII of 40 CFR Part 261)

One of the characteristics used is toxicity, which was previously defined according to the extraction procedure (EP). This test evaluates the likelihood of any of 14 toxic contaminants leaching from the waste placed in landfills. A revision of the EP, the toxicity characteristic leaching procedure (TCLP), allows 29 additional waste constituents, mostly chlorinated compounds and solvents, to be analyzed. The TCLP defines toxicity on the basis of substance concentration (mg/L). The added compounds were chosen primarily on the basis of available chronic toxicity reference levels (RfDs for noncarcinogens and RSDs for carcinogens), coupled with fate and transport data.

Wastes are placed on the federal listing if they contain substances that have been shown to have "toxic, carcinogenic, mutagenic or teratogenic effects on humans or other life forms." Waste may also be listed if acute exposure in low concentrations is likely to produce lethality (acute LD_{50} is < 50 mg/kg, acute LC_{50} is < 2 mg/L, or dermal LD_{50} is < 200 mg/kg) or "is otherwise capable of causing or significantly contributing to an increase in serious irreversible, or incapacitating reversible, illness."

Hazardous Waste (Subtitle C Program). This program tracks hazardous waste from cradle to grave, and sets standards for hazardous waste treatment, storage, and disposal facilities (TSDFs). With few exceptions, TSDFs must obtain permits; to do so, they must meet different sets of standards for containers, tank systems, surface impoundments, waste piles, land treatment, landfills, and incinerators. Ideal handling of hazardous wastes results in complete disposal; standards become particularly important, however, because treatment/disposal is typically incomplete. For example, incineration results in products of incomplete combustion (PICs), which are either released into the environment or remain at the facility for subsequent management (e.g., as ash).

Permitting hazardous waste treatment facilities requires public hearings, which in turn catalyze an informal risk assessment process to inform the public of the risk posed by the site. Two features of these risk assessments make them unique: the potential for multimedia exposures and the potential for exposures to chemical mixtures.

Nonhazardous Waste (Subtitle D Program). The Subtitle D rule, proposed in 1988, will include requirements for facility design and ground-water monitoring. As with hazardous waste, incineration (municipal waste combustion) is becoming an increasing popular treatment method.

3.6.1 Office-Specific Research Needs

3.6.1.1 Waste Characterization

Health data are needed to ensure that wastes are properly characterized. In the context of the risk assessment paradigm, this is a hazard identification problem. Of particular value would be:

- Methods for rapidly characterizing acute toxicity of waste
- RfDs for more noncarcinogens
- RSDs for more carcinogens
- A procedure for developing RSDs for noncarcinogens

3.6.1.2 Hazardous Waste

Data are needed to improve the quality of the informal risk assessments performed. In particular, research is needed to improve risk assessments that take into account:

- Multimedia exposures
- The potential for exposures to chemical mixtures

3.6.1.3 Nonhazardous Waste

Before siting a municipal waste combustor (MWC), the associated risk must be assessed (though EPA uses an informal process for these assessments). As with the Hazardous Waste Program, health research can improve these risk assessments. The risk from MWC emissions comes from both primary exposure to emissions and from secondary exposure"for example, through the food chain. Specific health research needs include:

- Determining the bioavailability of incinerator emissions via the various exposure pathways
- Evaluating the toxicity of the mixtures of PICs that characterize incinerator emissions
- Evaluating the comparative potency approach to performing risk assessments on such complex mixtures as incinerator emissions

3.6.2 Research Plan

3.6.2.1 Waste Characterization

Work in this area falls under the category of hazard identification. Four research needs were identified in Section 3.6.1; of these, the first (methods for rapidly characterizing acute toxicity of waste) depends most directly on laboratory research. The other three are best met by reviewing and modeling existing data. Research efforts in this area, therefore, are concentrated on improving methods for rapidly categorizing the acute toxicity of hazardous wastes. This effort, which will be relatively small, will begin by pinpointing the research needs and then will address those needs to the extent that they meet the criteria listed above. Endpoints that may be investigated include the use of *in vitro* tests for determining cytotoxicity as well as cytotoxicity as a predictor of acute toxicity.

3.6.2.2 Hazardous Waste

Work in this area falls under the category of dose-response assessment. These research efforts are designed to improve the health database from which risk assessments are performed. Risk assessments, which are performed through an informal process, must take into account:

- Multimedia exposures and the relationship between environmental concentration and dose to the target for each exposure route (to evaluate the dose-response relationship through pharmacokinetics and dosimetry)
- Pollution mixtures, particularly of metals

Thus, hazardous waste research is focused on these topics.

Because hazardous wastes are often deposited on soil, research efforts must consider the possible contribution of exposure through soil ingestion"a route of exposure that is particularly important for children. In addition, many compounds (e.g., lead) are known to be more toxic to children than adults. Presently, 100 percent of toxicants (e.g., metals) is assumed to be absorbed from soil. Consensus of opinion suggests that this assumption is invalid; but no agreement has been reached on how it should be modified. Furthermore, no agreement exists on the extent to which age affects bioavailability of substances from the gut.

In the short term, research efforts will address these issues, with particular emphasis on metals. In the longer term, work will concentrate on two related pollutant mixture issues: 1) how the presence of metals affects the absorption of other metals, and 2) the extent to which the assumption of additivity of risk is valid for metals. This is a new research program that will take shape following an effort to identify and prioritize the knowledge gaps.

3.6.2.3 Nonhazardous Waste

Research in this area falls under dose-response assessment. Research efforts will evaluate combustion emissions using the comparative potency approach. This approach may be viewed as a form of dose-response assessment for pollutant mixtures, though efforts to develop and validate the method fall under hazard identification work. The link between *in vitro/in vivo* bioassay data and human cancer following exposure to exogenous agents has been demonstrated for certain complex emission sources (e.g., roofing tar, cigarette smoke condensate, coke ovens, and open-fire smoky coal emissions) and lung cancer. Because incinerator emissions are likely to contain the kinds of chemicals found in these other complex emissions, the focus of research is on evaluating the relative potency of MWCs compared to other combustion sources. The research will place MWC emissions into a comparative potency framework with other combustion emission sources (e.g., hazardous waste incineration, hospital waste incineration). Based on these studies, OHR will be able to provide the Agency with advice on how (or if or when) to use the comparative potency approach to evaluate the health risk of complex mixtures.

In addition to predicting effects, risk assessments must also evaluate the dose of emissions that individuals are likely to receive. While modeling and monitoring efforts may allow prediction of ambient concentrations, additional tools (e.g., biomarkers) are necessary to estimate the dose. This research strategy, therefore, includes a small-scale effort to develop exposure biomarkers that can be used to estimate dose in a complex mixture context.