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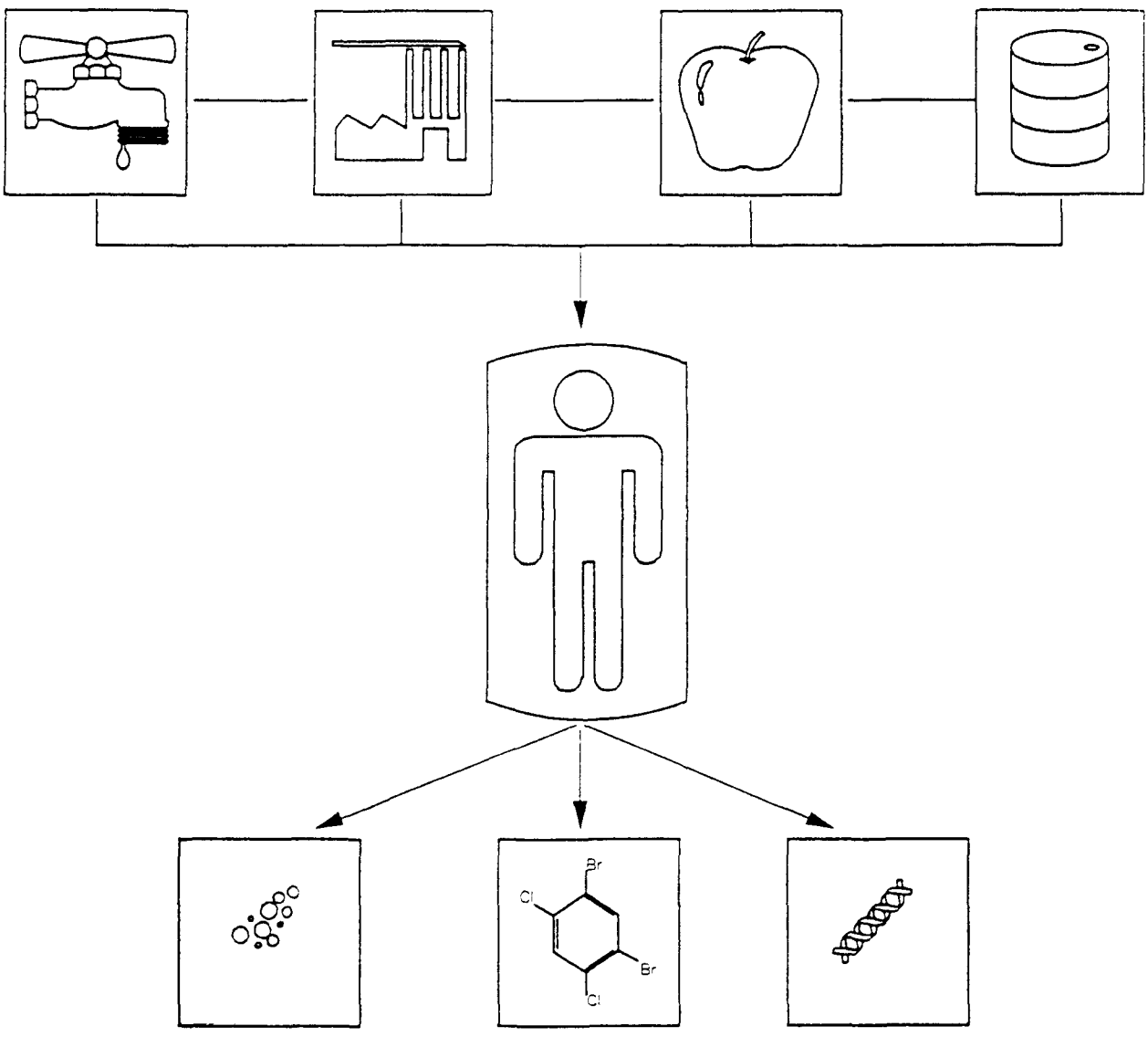
ORD Health Biomarkers Program

Research Strategy Document



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ORD HEALTH BIOMARKERS PROGRAM

Research Strategy Document

by

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Preface

Targeted environmental health research, including both basic and applied research, is needed if the Environmental Protection Agency (EPA) is to evaluate accurately the relationship between environmental exposures and human health risks. Over the past few years, Office of Research and Development (ORD) scientists have worked to define how "Biomarker" measurements made on human tissues, fluids and excreta, can be used to improve the assessment of environmental health risks. This document is the product of their efforts.

Initially, ORD co-sponsored a project with the National Academy of Sciences to develop biomarker concepts and definitions, and to identify which markers are available for use now and which need additional research before they can be applied to human populations. Based on the results, ORD developed a draft research strategy that was reviewed by the Environmental Health committee of EPA's Science Advisory Board. Their suggestions helped to make this a better document.

The import of the research described in this document goes beyond ORD. The terms and concepts were recommended by the International Programme for Chemical Safety (IPCS) as a model for using biomarker data to evaluate health risks.

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Disclaimer

This document has been subjected to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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List of Acronyms

ATSDR	Agency for Toxic Substances and Disease Registry
CAA	Clean Air Act
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CSF	Cerebrospinal fluid
CWA	Clean Water Act
DNA	Deoxyribonucleic acid
EPA	Environmental Protection Agency
ETS	Environmental tobacco smoke
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FR	Federal Register
HERL	Health Effects Research Laboratory
HIS	Health Interview Surveys
HIV	Human immunodeficiency virus
LOAEL	Lowest-Observed-Adverse-Effect Level
LH	Luteinizing hormone
MDSD	Monitoring and Data Support Division
NAAQS	National Ambient Air Quality Standards
NAL	Nasal lavage
NAS	National Academy of Sciences
NHANES	National Health and Nutrition Examination Surveys
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NO_x	Nitrogen oxide
NOAEL	No-Observed-Adverse-Effect Level
ODW	Office of Drinking Water
OHEA	Office of Health and Environmental Assessment
OHR	Office of Health Research
OMMSQA	Office of Modeling, Monitoring Systems and Quality Assurance
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OTS	Office of Toxic Substances
OWEP	Office of Water Enforcement and Permits
PCBs	Polychlorinated biphenyls
PHS	Public Health Service
RCRA	Resource Conservation and Recovery Act
RIHRA	Research to Improve Health Risk Assessment
SCE	Sister Chromatid Exchange
SDWA	Safe Drinking Water Act
TEAM	Total Exposure Assessment Methodology
TSCA	Toxic Substances Control Act

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Section One
Introduction

1.1 OVERVIEW

The U.S. Environmental Protection Agency (EPA) is responsible for protecting public health from the adverse effects of exposures to environmental agents. In developing regulations to protect public health, EPA currently relies on quantitative assessments of the health risks associated with different pollutants. However, the current lack of understanding about the underlying biological, chemical, and physical processes that determine exposures and effects hobbles EPA's ability to make these assessments. Often, quantitative risk assessment is entirely precluded because of the paucity of appropriate data. In other cases, risk assessment is possible, but numerous assumptions representing "fall back" or "default" positions must be applied due to critical data gaps. Application of these assumptions fosters enough uncertainty regarding the interpretation of the available data that diametrically opposed risk assessments can be made on the basis of the same information by groups employing different default positions.

A stronger scientific basis for risk assessment is the key to EPA's making more informed decisions for safeguarding public health. The current revolution in molecular science has acted as a driving force in the development of this fundamental framework. Over the next decade, an avalanche of biologic measures bearing on chemical exposure and human disease is likely to flow from these scientific advances. Chemical engineers, for example, are developing ever-more-sensitive techniques for measuring chemical substances and are coupling these techniques to sophisticated instrumentation capable of detecting minuscule amounts of substances, even in chemical mixtures such as blood and other body fluids. With such techniques, scientists can explore life processes at the most elemental levels — for example, how proteins control the formation of other components of cells.

In addition, major advances in toxicology and clinical/occupational medicine are fostering a better understanding of the biological, chemical, and physical processes that are important in maintaining health and in responding to chemical and other challenges. One of the most important of these advances is an increased understanding of the human genome, which molecular geneticists and other scientists are well on their way to mapping within the next 15 years. The fruits of this achievement could be staggering: once scientists can pinpoint the genes that cause various diseases such as some forms of cancer, cystic fibrosis, and Down's syndrome, they may be able to alter the course of the disease or to eliminate it entirely.

This scientific revolution is fueling the identification of biological markers, which are thought to offer great promise in reducing the uncertainties associated with risk assessment. "Biomarkers," as defined by the National Academy of Sciences (NAS, 1989a), are indicators of variation in cellular or physiological components or processes, structures, or functions that are measurable in a biological system or sample. For example, a high blood lead level is a good indicator of human exposure to lead and can be measured by laboratory sampling. Ultimately, biomarkers will be used as essential tools in monitoring and controlling environmental exposure to a broad range of contaminants.

This document outlines the framework for developing, validating, and applying biomarkers that ORD uses to facilitate planning, budget allocations, and collaboration in biomarker research. As a framework for biomarker efforts in ORD, this document is "a plan for a plan." Within the framework, ORD evaluates EPA's regulatory needs, its own capabilities, and the state-of-the-science. During this evaluation, ORD considers biomarker techniques as tools in understanding life processes; thus, rather than exploring biomarkers as ends in themselves, ORD incorporates biomarker research efforts into ongoing and future research programs. Note that the research efforts described in this strategy document cover only biomarkers for human health effects, though ecological biomarkers can also be identified. The document also defines terms and concepts used in this research in an effort to standardize their use across ORD laboratories, in keeping with the Total Quality Management philosophy adopted by EPA.

The ORD biomarker research program will position EPA at the forefront of biomarker research, thus allowing the Agency to take full advantage of the new technology as it evolves. As part of this positioning, ORD must examine many scientific and ethical issues raised by biomarker research. For example, how will ORD recruit and employ molecular biologists, as well as retrain chemists, biologists, and engineers? What priorities will the Office set for work both in the near and longer term? How will advances in science affect the traditional roles of ORD offices (distinctions will tend to blur as work takes on a greater molecular emphasis)? What implications do scientific advances over the next decade hold for the way ORD does business?

Also, the increasing understanding of the genome raises a number of difficult ethical issues. People with certain genetic traits may have difficulty obtaining jobs or getting insurance because of their susceptibility to sickness or death. In addition, individuals carrying genes for diseases for which no successful intervention procedure exists may suffer great mental anguish. (For more information on these concerns, see Section 2.3.4.)

Besides this document, other strategy documents have been or are being written. They discuss and document specific ORD plans for developing, validating, and using human biomarkers within ongoing ORD program areas. These include:

- Cell Receptor-Xenobiotic Complexes as Exposure Biomarkers
- Decision Model for the Development of Biomarkers of Exposure
- Strategy for OMMSQA Biomarkers Research Program
- Human Exposure Research Program: Strategy and Plan
- Protein Adduct-Forming Chemicals for Exposure Monitoring: Literature Summary and Recommendations
- Protein Adduct-Forming Chemicals for Exposure Monitoring: Chemicals Selected for Further Study
- Selection of Adduct-Forming Chemicals for Human Monitoring Studies
- Role of Pharmacokinetics and Biomarkers in Risk Assessment Implementation Plan: Part I. Exposure Assessment
- Research to Improve Health Risk Assessments (RIHRA) Program (Topic III. Physiologically Based Pharmacokinetic Models)

1.2 ORGANIZATION OF THIS DOCUMENT

The remainder of Section One defines terms used by ORD to describe biomarker efforts and outlines the framework for risk assessment as well as project office biomarker needs. Section Two discusses ORD's strategy for the biomarker research program, outlining the criteria established for selecting projects and highlighting which research areas have been given first priority for biomarker development. One of the program's selection criteria is to identify and take advantage of *current* opportunities to develop, validate, and apply biomarkers in human populations. Section Three describes the program's philosophy for using currently available exposure and effects biomarkers in near-term human studies. Section Four presents the program's directions for longer-term research to identify and develop new biomarkers.

1.3 CASCADE OF EVENTS BETWEEN EXPOSURE AND DISEASE

Understanding the human health risk associated with environmental exposures involves defining the cascade of events between exposure to an environmental agent and resulting health effect. To cause a health effect, a pollutant and/or its metabolite(s) must be absorbed into the body, reach a target organ, and result in a biological change. For many environmental pollutants, little is known about this flow of events between exposure and health effect. Biomarkers have the potential for shedding light on the factors influencing how much of a pollutant is absorbed and reaches a target organ and the resulting biological effect of that target dose.

Figure 1 shows the cascade of events between exposure and disease. The squares represent potentially measurable events that may be defined by biomarkers. Biomarkers can be structurally classified by the point in the cascade at which a measurement is taken (vertical arrows, Fig. 1):

Exposure - indicators of absorbed or target dose. An absorbed pollutant, its metabolite(s), or products resulting from interaction with endogenous substances measured in a body tissue or fluid is a biomarker of exposure. These biomarkers, such as blood or urinary lead, provide information concerning the *chemicals* to which human populations are exposed. Most exposure biomarkers are indicators of absorbed dose.

Effect - indicators of biological effect. These biomarkers provide information concerning the likely *health outcomes* associated with different target doses of environmental pollutants or metabolite(s).

Susceptibility - indicators of whether the individual or the subpopulation is more or less sensitive to exposure to a particular environmental pollutant. Sensitive subpopulations, for example, can be pinpointed by an increased absorption rate or a more severe biologic response.

These and other important terms in biomarker research are defined in Table 1-1.

Gold Standard

Ideally, sufficient information would be available on the links between events in the cascade so that any biomarker could be quantitatively related to either end of the spectrum. In other words, given enough information on the underlying processes, an "exposure" biomarker such as blood lead level could be used to quantitatively estimate both the exposure that must have occurred in the past and the likelihood of a specific health outcome in the future. For most environmental pollutants, the available data are and will be insufficient to allow such a broad application of a biomarker. However, ORD's research programs in pharmacokinetics (the effects of the duration, magnitude, and frequency of exposure on the

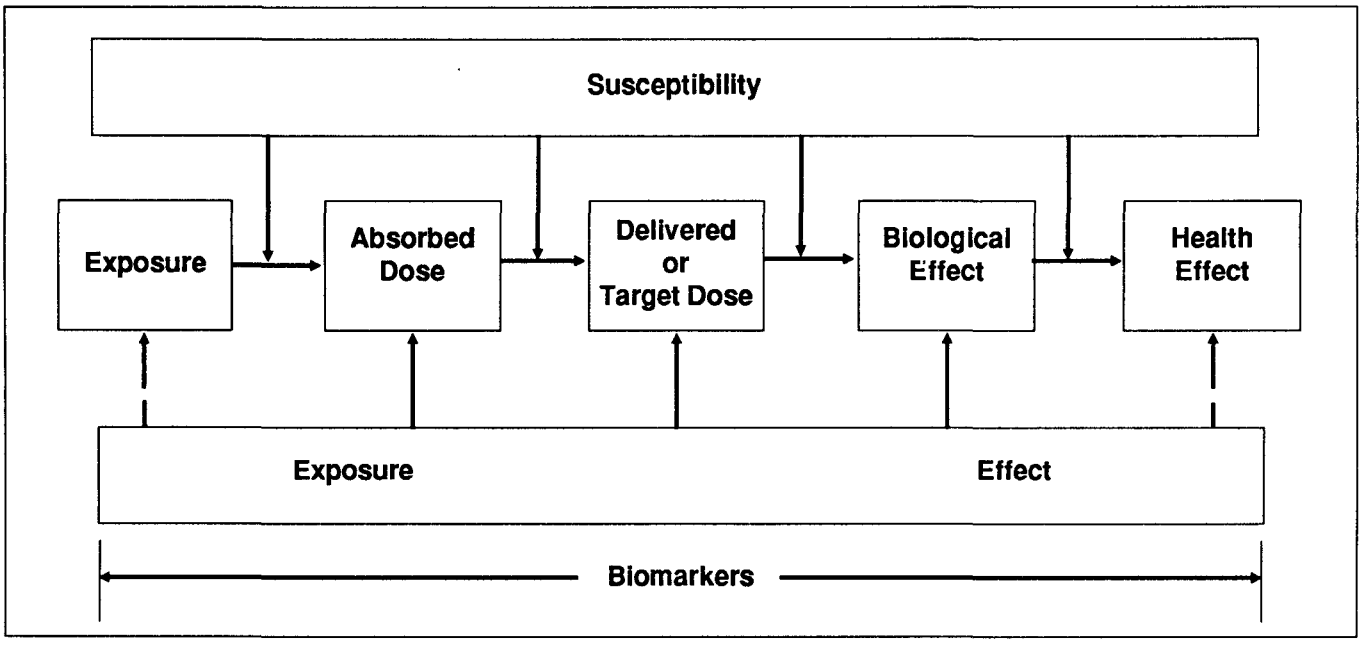

Figure 1. Utility of biomarkers as measures of exposure, susceptibility, and disease. The center row of boxes identifies key parameters in risk assessment. The upper and lower boxes identify how biomarkers can be used to provide data for assessing risk.

dose to the target organ) and dose-response (estimation of the incidence of a specific health effect in human subpopulations) are designed to help provide the necessary data on the middle steps in the cascade for priority biomarkers. These efforts will probably result in the development of an appropriate suite of biomarkers for evaluating the cascade from exposure to effect.

For general use, the Agency has narrowed the NAS definition of biomarkers (see p. 2) as follows:

> A biomarker must be taken from material from an intact organism or involve a functional evaluation of the organism itself, and the measurement must serve as an indicator of susceptibility, exposure, and/or effect.

ORD's health biomarker program further limits the definition of biomarker to "measures of *human* susceptibility, exposure, and/or effect." (A separate document, Ecological Biomarker Strategy for Research and Development, defines the terms and concepts ORD will use in developing, validating, and applying biomarkers for ecological effects evaluation (U.S. EPA, 1990g).)

1.4 RISK ASSESSMENT FRAMEWORK — THE SCIENTIFIC RATIONALE FOR BIOMARKER RESEARCH

Estimating the risk associated with exposure to a given pollutant involves evaluating, for that chemical or chemical class, the likelihood of resulting health outcomes and themagnitude of such effects (i.e., does exposure result in a cascade of events leading to health effects?). To develop these estimations, EPA has adopted a formal risk assessment

TABLE 1-1. Key Structural Definitions in Biomarker Research

Term	Definition
Concentration	Amount of material (contaminant) per unit of volume or mass in an environmental sample
*Exposure	Contact between an environmental contaminant and a living organism(s) (e.g., human, indicator organism, ecosystem); this may result from a single challenge or from contact at a given concentration over time
*Absorbed dose (Internal dose)	Amount of material that crosses one or more of the body's boundaries; often absorbed dose is best measured by the area under the curve of intake versus time
*Delivered dose (Target or biologically effective dose)	Amount of the absorbed dose and/or its metabolites that reaches the target (e.g., tissue, cell); often, delivered dose is best measured by the area under the curve of tissue concentration versus time
Body burden	Amount and distribution of material and/or its metabolites in the body
Biological effect	A measurable response in a molecule, cell, tissue or fluid
Health effect	A biological effect that causes dysfunction, injury, illness, or death
Susceptibility	Increased or decreased resistance to absorption of and/or effect from chemical substances due to genetic predisposition, environmental or lifestyle factors

*Time component is not always critical.

process, the steps of which mirror this flow of events. This process is based on recommendations from the National Research Council of the National Academy of Sciences (NAS, 1983). Given the inadequacy of current databases, these steps also represent critical questions in environmental health research:

- **Exposure assessment** - What exposures occur or are anticipated to occur in human populations?
- **Hazard identification** - Is the agent capable of causing an adverse effect?
- **Dose-response assessment** - What is the quantitative relationship between dose and effect in humans?
- **Risk characterization** (based on syntheses of dose-response and exposure assessments) -What is the estimated health risk (occurrence/magnitude) from the anticipated human exposures?

The use of biomarkers can improve the risk assessment process. First, the development and analysis of biomarkers provides data needed to construct the scientific framework for risk assessment. Using biomarkers to monitor humans for evidence of environmental exposure and compromised health will highlight the factors involved in how chemical substances enter the body, reach target sites, and elicit biologic responses. Second, biomarker analysis can make the risk assessment process more realistic and more cost-effective. For example, the level of a chemical or its metabolite(s) in the blood is irrefutable evidence of exposure; and, given data on the kinetic behavior of the chemical in the body, risk assessors can accurately estimate the human exposure to the chemical from that biomarker. This estimate can be made without relying on traditional assessment techniques, including the use of assumptions based on data from laboratory test species.

When enough is known about the cascade of events following exposure to a pollutant, a biomarker can be used to estimate both level of exposure and probable outcome. Blood lead and carboxyhemoglobin levels can currently be used in this way to link exposure to health effect. From a practical perspective, however, such relationships will be known for very few biomarkers. Thus, combinations of biomarkers need to be developed for use together in evaluating risk.

Trends in biomarkers can also suggest changes in exposure level. These measurements, when compared with the level of the biomarker in control populations, can provide strong evidence of environmental contamination or of exposures in an occupational setting. In addition, when these biomarker trends can be associated with a specific environmental change, risk assessors and risk managers can use these data to catalyze changes in environmental policy or regulations.

1.5 KEY RESEARCH ISSUES

The critical research questions for improving human health risk assessments (see Section 1.4) underlie the research needs of all EPA regulatory program areas. These questions can be further subdivided into six long-standing research issues that cut across environmental media, scientific disciplines, pollutant classes, and regulatory programs. Handling these issues will require both basic and applied research as well as a substantial commitment of time, effort, and resources. Biomarkers, as indicated below, can be important tools in facilitating research addressing these different issues.

- **Exposure Assessment Research.** Populations are exposed to a wide spectrum of chemicals. These environmental contaminants may enter the body through inhalation, ingestion, and/or dermal absorption. Exposure may be episodic (e.g., a chemical spill), intermittent, short-term chronic (e.g., rush hour traffic), or long-term (e.g., contaminated drinking water). Risk assessors need better information on actual human exposures, including magnitude, duration, frequency, and route of exposure. Researchers need to improve sampling and analytical methods to design and carry out population-based exposure measurement programs and to develop and validate appropriate exposure models. **Biomarkers are needed that provide a memory of exposure from multiple routes or times.**
- **Hazard Identification Research.** To identify potential health hazards, EPA needs validated, short-term test methods (both *in vitro* and *in vivo*) for screening new and existing environmental agents. Such tests will allow risk assessors to determine in a timely manner whether an environmental agent causes an adverse health outcome. Agents of current concern include biotechnology products released into the environment, alternative fuels (e.g., methanol), and increased ultraviolet radiation due to stratospheric ozone depletion. **Scientists need effect biomarkers to identify exposures and possible health outcomes.**
- **Dose-Response Research.** Because of the limitations of current databases, risk assessors must usually estimate human response to a pollutant from data gathered through exposing laboratory animals to high pollutant exposures. The resulting effects must then be extrapolated to a different species and to different exposure conditions, because humans are usually exposed to low levels of pollutants and experience chronic rather than acute effects. Large uncertainties are associated with this extrapolation process. Improving the accuracy of risk assessments requires a better understanding of the physiologic and biochemical mechanisms of toxicity, including compensatory processes. **In this area, researchers need homologous**

markers that can be used in different species to link exposure to disease.

- **Risk Characterization Research.**

Cancer and Noncancer Health Effects. A variety of health outcomes may arise from an environmental exposure. In addition to carcinogenicity, adverse health consequences may include various systemic (e.g., pulmonary, cardiovascular) effects. Noncancer health risk assessment involves determining which health outcomes are important, for which environmental contaminants, and under what conditions. The Agency currently relies on a "safe dose" approach to noncancer effects (e.g., reference doses, maximum concentration limits). However, developing an appropriate methodology for quantitative risk assessment is critical for improving risk management decisions in this area. To do so, scientists must establish criteria for defining the adversity and severity of effects and take into account the different effects induced in multiple organ systems. **Batteries of markers that could be studied in parallel at different points in time following a challenge would be key tools in these efforts.**

Chemical Mixtures. Humans are usually exposed to environmental contaminants in mixtures rather than singly. Examples include emissions from hazardous and municipal waste incinerators, urban air pollution, and drinking water contaminants. Historically, *in vitro* and *in vivo* tests have been developed to establish which components of a mixture are most hazardous and to compare the health risks of different mixtures. Now, researchers must determine whether the health risks of a pollutant mixture can be reasonably assumed to be equivalent to the sum of the risks associated with the specific mixture components. **In this area, markers could be used to determine the relative importance of exposure with respect to different pathways (e.g., air, water, soil) and disease outcome. Markers can also be used to determine if exposure to multiple chemicals results in additive effects.**

Evaluation of Human Populations. Obtaining exposure, dose, and effects data in human populations is key to assessing the status of public health, identifying potential problems, and evaluating the efficacy of risk reduction measures. This process is also important for identifying and safeguarding population subgroups that may be at elevated risk because of either increased susceptibility or higher exposures. Human data are also needed to assess the comparability of effects observed in animals and people. **Researchers need noninvasive, inexpensive, informative markers for gathering exposure and effects data from human subjects.**

1.6 SPECIFIC PROGRAMMATIC NEEDS — THE REGULATORY RATIONALE FOR BIOMARKER RESEARCH

EPA'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 (CAA); Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Toxic Substances Control Act (TSCA); Clean Water Act (CWA); Safe Drinking Water Act (SDWA); Resource Conservation and Recovery Act (RCRA); and the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), require that EPA develop regulatory programs to protect public health. In performing this task, EPA must analyze the toxicological effects of specific pollutants in exposure media (e.g., air, water, soil, food, sludge) as well as develop health risk assessments. Most assessments to date are based on data from animal toxicology studies or human epidemiologic studies and estimates of environmental exposure. Because they are tools for pinpointing exposure, susceptibility, and effect, biomarkers are used to support the chemical-specific needs of the program offices as well as the longer-term establishment of a stronger scientific basis on which to build health risk assessments.

This section highlights current uses of biomarkers in support of the EPA program offices as well as potential future applications.

1.6.1 Clean Air Act

Current uses of biomarkers to support CAA: Low levels of alpha-1-trypsin in the blood may indicate incipient or existing emphysema. Researchers also use blood lead and carboxyhemoglobin to indicate exposure to lead and carbon monoxide, respectively, as well as decrements in lung function to identify exposure to ozone.

Potential uses of biomarkers: Pollutant-specific markers could be used for monitoring air pollution trends on a local and national level and as early warning signs for asbestos toxicity, emphysema, and lung cancer. Biomarkers can also serve as tools for epidemiological research — especially biomarkers for developmental neurotoxicity, immunotoxicity, and reproductive toxicity, which can be used to assess physiologic responses of the lung and to provide physiological measures of effect for victims of the sick-building syndrome (due to polluted indoor air). Refining information on cotinine in urine as a biomarker for exposure to environmental tobacco smoke (ETS), for example, is much needed.

1.6.2 Federal Insecticide, Fungicide, and Rodenticide Act

Current uses of biomarkers to support FIFRA: The Office of Pesticide Programs (OPP) is using biomarkers now in risk assessments on pre-registered pesticides. The office uses "classical" biomarkers such as measurement of cholinesterase inhibition, liver enzyme changes, DNA adducts, and repair synthesis.

Potential uses of biomarkers: Markers for exposure, reproductive function, genotoxicity, cancer, and pulmonary effects could be used, as well as markers for outcomes associated with specific classes of pesticides that can be used to evaluate whether humans respond to various levels of pesticide exposures. Such information would be used to refine tolerance levels and evaluate human safety.

1.6.3 Toxic Substances Control Act

Current uses of biomarkers to support TSCA: The Office of Toxic Substances (OTS) is using biomarkers to measure national trends of pesticide and other chemical exposures. Measurements of fat and blood levels for these chemicals are being used to assess uptake on a national basis.

Potential uses of biomarkers: The development of more precise biomarkers would support the refinement of national exposure and uptake assessments. Biomarkers of effect would provide valuable information for extrapolating animal test data to human outcomes. In particular, biomarkers that are predictive of neurotoxic endpoints and carcinogenesis are needed.

1.6.4 Clean Water Act

Current uses of biomarkers to support CWA: Biomarkers are being used in water quality advisory and sludge criteria development (e.g., cholinesterase inhibition). The water quality criteria are being developed on the basis of endpoints such as lethality, reproductive impairment, carcinogenicity, mutagenicity, and teratogenicity.

Potential uses of biomarkers: The Office of Water Enforcement and Permits/Monitoring and Data Support Division (OWEP/MDSD) needs biomarkers as screening tools to detect human and fish exposures to pollutants originating in industrial/municipal effluents. Tests are sought that are quick, inexpensive, and easily traced to a point source. As an example, pollutant levels could be measured in blood or urine to pinpoint a single industrial point source which discharges to a river used for recreation, drinking water, or fish consumption.

1.6.5 Safe Drinking Water Act

Current uses of biomarkers to support SDWA: Many drinking water standards are based on exposure estimations based on biomarker measurement, such as No-Observed-Adverse-Effect Levels (NOAELs) or Lowest-Observed-Adverse-Effect Levels (LOAELs). Examples of chemical classes for which these have been prepared include organophosphate pesticides (biomarker: absence of cholinesterase inhibition); metals (effect: liver or kidney damage; biomarker: level of blood or urinary enzymes, blood lead level); and chlorinated disinfectants (effect: long-term chronic; biomarker: serum cholesterol levels, effects of immune toxicity).

Potential uses of biomarkers: Biomarkers will continue to be used to implement regulations under the Office of Drinking Water (ODW). As with other programmatic areas, a key issue in using biomarkers to develop drinking water standards and advisories is ensuring that a clear cause-and-effect link can be made between the biomarker and exposure from a specific chemical.

1.6.6 Resource Conservation and Recovery Act

Current uses of biomarkers under RCRA have not been identified. In the future, however, biomarkers are needed in two areas: hazard assessments under Sections 3013 and 7003, and as a tool to assess the national or local success of hazardous waste control and disposal management methods. For example, biomarkers could be used to define exposed populations in the vicinity of RCRA-permitted facilities, and to reassure enforcement staff that exposure is within an acceptable range. Also, these measurements could be used to assess the risks associated with treatment variances and special wastes from the oil and gas, coal-fired utility and cement industries, as well as medical wastes, incinerator emissions, and contaminated soil.

1.6.7 Comprehensive Environmental Response, Compensation and Liability Act

Current uses of biomarkers to support CERCLA: The Agency for Toxic Substances and Disease Registry (ATSDR) uses a variety of biomarkers in human monitoring studies at Superfund sites.

Potential uses of biomarkers: Exposure biomarkers are of more immediate interest to the Superfund program than effect measurements: Regulatory enforcement schedules cannot wait for the results of the longer-term research required to develop effects biomarkers and validate their predictive value. Biomarkers are needed for national and local trend analysis — for example, to assess whether people are being exposed to hazardous substances and, if so, by what routes. Of particular concern to the Superfund program are noncancer risk assessment methodologies, including genetic, nervous system, and reproductive system effects from chemical exposure and combinations of effects from exposures to complex chemical mixtures. Chemical-specific and endpoint-specific biomarkers are needed to provide remedial project managers with a more complete and realistic understanding of health risk.

To summarize, the key needs for the regulatory offices are cheap, quick, validated biomarker tools that provide data about exposure and key health effects, especially cancer, reproductive, and neurotoxic effects.

1.7 APPLICATION OF BIOMARKERS

The risk assessment and toxicological needs of the various program offices point to the connections between fundamental research, toxicity testing, and biomarker work. For example, biomarkers that can be used to evaluate exposures as part of EPA risk assessments are often tools used in mechanistic studies on membrane integrity, transport processes, and disease mechanisms. Thus, research to develop physiological models is closely integrated with efforts to develop risk assessment models.

Biomarkers can be used in field studies examining a variety of specific test populations. These efforts cover:

- Spills/accidents — situations that present opportunities to study short-term exposures to atypically high concentrations of chemicals.
- Clinical drug testing — carefully controlled, measured exposures to specific substances.
- Pollutant testing — controlled exposure studies to a known dose of a substance, such as ozone or NO_x.
- Case studies of disease incidence — cases in which health effects are known or suspected, but the cause(s) of the problem are unclear.
- Occupational cases — situations in which the disease incidence appears to be related to a common occupation, or the suspect chemical substance/mixture is peculiar to an occupational setting.
- Chronic nonoccupational — low-level exposures over long periods of time, such as residents downwind of an industrial facility or waste site.
- Status and trends — work to identify trends in a large population at discrete points in time, possibly over a period of years.

The potential usefulness of biomarkers in such field studies is the primary reason the Agency is supporting research to develop, validate, and apply these measurement tools. For more detail, see Section 3.1.

Section Two
Biomarker Research Strategy

This document focuses on the issues and priorities involved in establishing an ORD biomarker strategy rather than on the specific scientific approaches used to implement that strategy. For documentation concerning ORD's plans for using biomarkers, refer to the following documents: Role of Pharmacokinetics and Biomarkers in Risk Assessment Implementation Plan: Part I, Exposure Assessment (EPA, 1990e); the Human Exposure Research Program: Strategy and Plan (EPA, 1990b); and the OMMSQA Exposure Research Strategy (EPA, 1990a). In this section, the key criteria for selecting ORD biomarker research projects are outlined and key issues are introduced.

2.1 ROLES OF PRIMARY ORD OFFICES

Extensive planning and coordination among the ORD offices will be required to implement an ORD-wide biomarker program. Three offices will play key roles in this effort. The Health Effects Research Laboratory (HERL) in the Office of Health Research (OHR) has the lead in conducting laboratory research and developing the biology for the biomarker methods as well as in developing the associated experimental data needed to develop the PB-PK models. The Office of Modeling, Monitoring Systems and Quality Assurance (OMMSQA) has the lead in validating exposure biomarkers through the development of measurement devices and exposure models and the gathering of human field data (in collaboration with HERL epidemiologists). The Office of Health and Exposure Assessment (OHEA) has the lead for risk assessment applications and will work closely with OMMSQA and HERL scientists to develop risk assessment models that synthesize exposure, pharmacokinetics, and effects data. These efforts help identify data and model limitations, which can then be addressed in subsequent research projects.

2.2 ESTABLISHING A BIOMARKER RESEARCH STRATEGY — CRITERIA AND PRIORITIES

The criteria ORD will apply in selecting biomarker projects are described here in priority order.

I. Overarching Criteria

Projects must:

1. Address the major scientific uncertainties in risk assessment:

   —High-to-low-dose extrapolation

   —Dose rate effects on tissue damage

   —Nonlinearities in pharmacodynamics

   —Validating route-to-route extrapolation

   —Elucidating mechanisms of toxicity

   —Explaining species, sex, and/or strain differences

   AND/OR

2. Provide chemical-specific data needed by regulatory programs, either in current or future standard-setting activities.

   The biomarker program will give first priority to projects designed to advance knowledge on key issues in risk assessment. To the extent possible within that context, projects will be selected that address chemicals of concern to program offices. Any project that addresses a scientific uncertainty in risk assessment through work on a chemical of great regulatory concern will be given top priority in the program.

   Key scientific issues for risk assessment:

   —To what chemicals are people exposed?

   —What amount of a chemical is absorbed into the body across the skin, lung, or gut barriers?

   —Where do chemicals move in the body and what happens to them there? How does their fate depend on the route of entry?

   —What early biological responses occur following exposure to the target organ(s)? Which of these can be measured in the blood or urine?

   —How do these early responses relate to health outcome?

Prioritization of chemical-specific efforts:

Chemical-specific projects will be selected on the basis of these factors associated with a chemical:

—Frequency of occurrence in the environment

—Uniqueness of occurrence in the environment as opposed to occurrence in medicines, the workplace, or food

—Physical and chemical properties

—Toxicological properties

—Chemical specificity

—Significant human exposure

3. Provide near-term payoffs (i.e., short-term applied research and longer-term applied fundamental research with interim outputs).

Work on exposure biomarkers will be given first priority because these efforts will have the greatest near-term, practical impact on improving risk assessments. Questions these studies can answer include: To what chemicals is a population (individual) exposed? To how much of a particular chemical is a population (individual) exposed? Exposure biomarkers are also often easier to develop and use than effects biomarkers. Exposure markers can be measured by simple tests (e.g., on blood, breath, and urine), while effects markers often involve techniques that are more invasive and expensive.

The state-of-science in a particular discipline, coupled with the ease of developing biomarkers in that area to achieve near-term payoffs, determine the prioritization of work on **effects** biomarkers. Projects on effect biomarkers represent longer-term research, focusing on such questions as: What is the target organ dose? What response is occurring in an exposed population (individual)? What are the steps from exposure to effect? The highest-priority disciplines for development of effects biomarkers are as follows:

—**Cancer.** Many advances have been made in this area, due to a strong research focus over the last decade. Emphasis will be placed on target organ and molecule dosimetry and on enzymatic and cell surface markers of neoplasia.

—**Pulmonary.** ORD has a sophisticated pulmonary program and extensive working knowledge of pulmonary function. Emphasis will be placed on validating techniques for use in clinical studies to characterize dose-response relationships and on developing noninvasive techniques for field studies.

—**Neurotoxicology.** This is an important regulatory endpoint and classical neurotoxicity techniques provide a means for validating newly developed techniques. Emphasis will be placed on validating dose-response relationships and on developing noninvasive techniques.

—**Reproductive.** Serum, urine, sperm, and sputum samples can provide insight into system function. Emphasis will be placed on techniques for assessing fertility.

—**Immunotoxicity.** Many measures are available. The emphasis will be on evaluating the health significance of these measures.

Within these priority areas, the program gives precedence to projects developing new measurement techniques either for which no alternatives are available or that are less expensive, quicker, and better than available techniques.

Second-priority topics include work in these disciplines:

—**Developmental.** Biomarker efforts in this area require invasive techniques. Furthermore, there are limited opportunities for studies of pregnant women following environmental exposures.

—**Heritable Mutation.** Presently available methodology is inadequate to demonstrate chemically induced heritable mutations in humans. Genetic variation is also an unresolved issue. Biochemical measures can be used to identify altered phenotypes.

—**Other Organ Systems.** Other agencies are focusing on this area. The Agency for Toxic Substances and Disease Registry (ATSDR) and the Centers for Disease Control (CDC), for example, are studying kidney and liver biomarkers (CEHIC/ATSDR, 1988).

Third-priority topic: Susceptibility

Much of the scientific basis needed to develop **susceptibility** biomarkers is still not available, and this type of biomarker also raises many ethical questions. For example, should individuals in a test population be told they carry a biomarker pointing to an increased likelihood of contracting a serious illness? Projects on susceptibility biomarkers score low on the third criterion for near-term results; however, to the extent that studies in various scientific disciplines develop data on susceptibility, this information will be applied to biomarker work.

For exposure, effects, and susceptibility biomarkers within each discipline, projects would be selected through a tiered approach appropriate to the state-of-the-science and the specific test population: Tier 1, quick, inexpensive, noninvasive studies designed to determine the presence of exposure or health effects;

Tier 2, more costly and invasive studies to confirm the initial observation and begin to focus on a particular aspect of either exposure or effect; and Tier 3, more narrowly focused and invasive examination to fully understand the exposure or effect. In the design of biomarker studies, Tier 1 tests will be relied on as much as possible. Animal studies and other nonhuman studies will be used as appropriate, but human studies will be needed to validate the Tier 1 tests. As opportunities arise for using clinical and other invasive Tier 2 and Tier 3 techniques to obtain human tissues, these will be evaluated on a case-by-case basis.

II. Importance of Flexibility: Response to Opportunity

4. Identify and take advantage of opportunities to develop, validate, and apply biomarkers in humans.

   The biomarker program must be flexible in its selection of projects. Opportunities to work with populations exposed to specific chemicals of interest are rare, and the program must have the capability to respond quickly to these chances. Section 3 discusses application of currently available biomarkers and details how further studies in test populations would apply these measures of exposure and effect.

III. Other Key Criteria

Three other key criteria are also used to prioritize biomarker projects. Selected projects must:

5. Provide a practical tool at a modest cost for work with human subjects for EPA's purposes.

   To be practical, biomarkers must be:

   —Measurable in breath, fluids, or tissues from humans.

   —Measured using non- or minimally invasive techniques.

   —Interpretable in terms of human susceptibility, exposure, and/or effect.

   —Indicate a significant and severe rather than a minor health effect.

6. Support competency building within ORD. Innovative projects and programs will attract top-notch scientists to EPA. Biomarkers competency building will be structured around pressing Agency biomarker needs.

7. Be leverageable with base program and other key programs, and must be integrated across ORD laboratories.

   Animal research conducted in the base and other programs is key to the development and validation of biomarkers (see IV below). Various ORD laboratories are involved in the development, validation, and/or application of biomarkers, and their efforts in exposure biomarkers are coordinated through a pharmacokinetics research program (see Section 2.3.1). In addition, biomarker efforts can vitally influence the success of other current ORD research initiatives, such as in epidemiology.

IV. Type of Study: Human as Opposed to Animal

Studies on exposure or effects biomarkers are performed in either 1) laboratory test species or 2) humans. Work on experimental animals is performed under the base research program or under a program called Research to Improve Health Risk Assessments (RIHRA) (EPA, 1990f). The biomarker program focuses on the development of measures (i.e., biomarkers) that can be applied in humans; RIHRA focuses on how to extrapolate risk from animal data to human exposures. As noted above in III 7, work conducted in the base program and RIHRA will be leveraged to develop human biomarkers to the fullest extent possible.

V. Type of Human Study

Biomarker work with human populations can be divided into three types of studies: clinical, epidemiologic, and field.

Clinical and epidemiologic studies will probably be conducted only for chemicals of major environmental importance, such as National Ambient Air Quality Standards (NAAQS) air pollutants (e.g., ozone, carbon monoxide) and water disinfectants or disinfectant byproducts (e.g., chloramine, dichloroacetic acid). Clinical studies are often invasive, expensive, and designed to be helpful in the treatment of individual patients. Patients who expect benefits from such markers will tolerate procedures they would not accept in the field. Epidemiologic studies can also be costly; however, biomarkers are a critical tool in improving exposure assessments in these studies, and efforts will be made to use ORD biomarker research to support any in-house exposure initiative as well as the work of other groups, such as the Centers for Disease Control, which runs large epidemiological studies.

Field studies, which are key to the validation of biomarkers, will emphasize status and trends monitoring and total exposure assessment or assessment of persons in chronic nonoccupational settings (e.g., near specific industrial sources or waste sites, or close to a chemical accident).

2.3 PROGRAM ISSUES

2.3.1 Coordinating ORD Research Efforts

As the state of environmental science has evolved over the past two decades, the distinctions between the missions of engineering, monitoring, ecological and health laboratories have blurred. Now, all the laboratories are integrated into a continuum (see Figure 1). Measuring the release of a chemical substance into the environment is not sufficient as a basis for sensible regulation. Decision makers also need information on human exposure, health risk, and the efficacy of control technologies. To efficiently use ORD resources, ORD laboratories need to collaborate, working to apply their expertise in part of the risk assessment continuum in concert with others to address the full array of risk assessment concerns.

The biomarker research conducted in various ORD laboratories must be forged into a unified, cost-effective program. ORD is composed of 14 laboratories scattered across the United States. Some of the laboratories have already developed independent biomarker research strategies within their respective disciplines. Furthermore, much research is currently underway in these laboratories in pharmacokinetics, a research area in which exposure biomarkers are a key tool. The first step in focusing these activities and determining lead responsibilities for exposure biomarkers was the establishment of an interlaboratory Working Group in January 1990. The coordinated pharmacokinetics strategy developed by this group will be phased in over the next two to three years, beginning in the 1990 fiscal year. The strength of the biomarker research program will be greatly enhanced by the continual exchange of ideas among the different laboratories and professional disciplines involved in biomarker research.

2.3.2 Leveraging the Research Results of Other Federal Research

Given its limited biomarker research budget, ORD will focus its efforts on pursuing unique and essential contributions to the technology for exposure and effects biomarkers. It will leverage the results of the very substantial research laboratory developments in biomarker assays that are already in progress in HERL, the National Institute for Occupational Safety and Health (NIOSH), the Agency for Toxic Substances and Disease Registry (ATSDR), the National Institute of Environmental Health Sciences (NIEHS), their extramural grants programs, and others, bringing the more promising biomarkers rapidly into field validations and applications. Early demonstrations of the utility and power of the emerging biomarker technologies will further stimulate additional research and applications in EPA programs and the field in general. To date, ORD had conducted literature evaluations of DNA adducts (EPA, 1990c), protein adducts (EPA, 1989c; 1990c), and chemically exposed populations (Uziel et al., 1989) to identify and assess the work done by others. (The chemically exposed population survey was conducted with the Oak Ridge National Laboratory.)

ORD has also taken advantage of opportunities to use the substantial databases from the National Health and Nutrition Examination Surveys (NHANES) and Health Interview Surveys (HIS). NHANES has large quality-assured databases on national population samples, including trace metals in blood, serum chemistry, tap water concentration, pulmonary function, clinical data, and household characteristics. These data, when tapped by ORD epidemiological research, show that highly significant associations can be made between high blood lead levels and elevated blood pressure in adults and reduced stature and hearing acuity in children. In addition, during the 1976-1982 NHANES II data collection interval, blood lead levels dropped precipitously, in parallel with the decline in lead content in motor vehicles fuel, thus showing the utility of blood lead as an exposure as well as an effects marker. Further use of the NHANES I and NHANES II databases will yield other linkages between environmental factors and human disease.

In addition, ORD commissioned the National Academy of Sciences (NAS) to define the state-of-the-science for biomarkers and to recommend which biomarkers are useful now and which show promise for future development. The ORD project officer established an advisory group of government experts to share information about their research programs and to advise the NAS panels. ORD heavily relied on these reports in the development of this document and is taking the results from the NAS studies (NAS, 1989a, b) into consideration in choosing research projects for funding.

2.3.3 Lack of Uniformity Among Scientific Disciplines

A third issue is the lack of uniformity in the development — and in the speed of development — of the different scientific disciplines associated with biomarker research. This variance is a factor in the types of studies that can be conducted. It also highlights a need to define the level of development in a discipline at which EPA should invest resources in biomarker research. To date, the state-of-science in each field has driven ORD biomarker research, and efforts have focused on animal studies to develop techniques for application in humans. However, current criteria for ORD biomarker research (see Section 2.2) place priority on programmatic as well as scientific considerations. The goal of the program is to develop the techniques most suitable for use in human studies that also meet EPA's regulatory needs.

2.3.4 Concerns Raised in Performing Studies on Human Populations

Performing biomarker studies with human populations raises both ethical and procedural issues. For example, what obligation does a researcher have to interpret the human health significance of a biomarker for members of a test population in whom the biomarker was identified? Principal investigators must clearly define the information that will be provided before the beginning of the study and ensure that all participants understand these conditions. Also, procedures should be established for the different types of techniques used to gather human data. These range from the use of discarded body fluid specimens obtained as part of routine medical care (e.g., human urine samples with no personal identifiers) to relatively invasive procedures performed specifically for a study (e.g., bronchoalveolar lavage or fat biopsy). These procedures must follow the guidelines established under 45 CFR 46 as amended under the pending Federal Policy for the Protection of Human Subjects (see 53 FR 218, Nov. 10, 1988). In addition, Institutional Review Boards must review all such procedures (with certain exceptions, such as the use of existing pathological specimens obtained without identifiers).

Special rules apply to human immunodeficiency virus (HIV) testing conducted or supported by the Public Health Service (PHS). Individuals whose test results are associated with personal identifiers must be informed of these results and given appropriate counseling, if requested. Exceptions to this policy are defined in the National Institutes of Health, "Office for Protection from Research Risks Reports," June 10, 1988.

For each study, EPA will develop a comprehensive plan for informing all participants of biological monitoring of the results. This plan will cover the issues of confidentiality and privacy. In addition, EPA needs guidelines for anticipating the ethical, legal, and social impacts of biological monitoring.

2.3.5 Validation of Biomarkers

Once a biomarker is developed, it must be validated. To validate a biomarker, researchers must show that the measurement can be used to reconstruct the level of exposure to a chemical or predict a target dose or resulting health effect. In addition, the regulatory offices require biomarkers that allow researchers to clearly identify the specific pollutants to which a population is exposed, or the specific routes of exposure involved (see Section 1.6). Validated biomarkers also provide a basis for extrapolating risk from animal data to human conditions of exposure. No biomarker will be used alone in a human study unless it has been validated.

Several ORD laboratories (see Section 2.1) will be involved in the validation of new biomarkers. In addition, animal data from research efforts in the base program will be used to supplement data obtained in biomarker field studies. Often, researchers will employ a putative biomarker in parallel with a well-defined measure(s) of susceptibility, exposure, and/or effect.

Section Three

Application of Current Biomarkers in Opportunistic Studies

If the opportunity arose now to develop or validate a biomarker in a human population exposed to a specific pollutant, what course of action would researchers in the ORD biomarker program follow? This section discusses how currently available exposure and effects biomarkers would be used in such studies over the next three to five years.

3.1 EXPOSURE BIOMARKERS

In establishing field studies of exposure biomarkers, ORD will critically evaluate the situation and design the effort accordingly on a case-by-case basis. No two studies are exactly the same. The kernel of the process, however, is provided below for two types of field studies — source-specific exposure modeling and status and trends monitoring.

Source-Specific Exposure Monitoring:

1. Answer the questions: What regulatory/enforcement or scientific issue requires exposure information? How will collection of biomarker data help the Agency?
2. Identify sources and quantify emissions to the environment.
3. Trace movement of chemical substances from source to human(s).
4. Screen the potentially affected humans with the aid of a questionnaire and measure the relevant validated biomarker of exposure, e.g., benzene in breath, blood lead, etc. If high, go to step 5 or 6.
5. Institute mitigation strategy. End sequence, except for follow-up if necessary.
6. Measure exposure pathways.
7. Measure personal exposure levels.
8. Relate the exposure levels, concentration in pathways and control group results.
9. If high, go to step 5. If low or further action is unwarranted, stop.

Current Status and/or Trends Monitoring:

1. Identify chemical(s) of top priority to EPA.
2. Identify exposed or potentially exposed human population.
3. Select appropriate validated biomarker.
4. Collect tissue/fluid/breath sample that will contain the biomarker.
5. Analyze sample for the biomarker presence and concentration.
6. Apply pharmacokinetic models to these biomarker data to estimate target tissue dose as well as exposure concentration.

3.2 EFFECTS BIOMARKERS

Similar to studies of exposure biomarkers, ORD will design effects marker field studies on a case-by-case basis. Again, no two studies will be exactly the same. Often, EPA will piggyback research onto human studies conducted primarily by others (e.g., CDC). The process ORD will follow is outlined below.

Effects Evaluation (with a validated biomarker):

1. Answer the questions: What regulatory/enforcement or scientific issue requires effects information? How will the collection of biomarker data help the Agency?
2. Identify chemicals of interest and their sources of exposure.
3. Review literature and other databases, using structure-activity relationship (SAR) techniques as appropriate to identify likely health out comes associated with exposure to the chemicals/sources identified in Step 2 based on known or presumed links between exposure and disease.
4. Select appropriate validated biomarker.

5. Collect tissue/fluid/ breath or other samples that will contain the biomarker.

6. Analyze the sample for biomarker presence.

7. Apply pharmacodynamic models to these data to predict health outcome.

When a human study is planned that incorporates proven techniques and ORD is using that opportunity to develop/validate a new biomarker for either exposure or effect, the following steps will be taken:

1. Identify chemical effects of concern.

2. Develop the concept — identify the likely consequence of chemical exposure that might serve as a useful measure of exposure.

3. Experimentally confirm validity of the concept by evaluating the basic mechanisms by which the biomarker responds to chemical exposure.

4. Develop method of measurement by identifying a method for detecting changes in the biomarker at doses at or below those producing toxic effects.

5. Determine if the biomarker is practical for the field by developing plausible field methodology and assessing the sensitivity of the biomarker in monitoring existing exposures.

6. Establish a dose-response relationship. Kinetics of biomarker response must have a rational relationship to metabolism and pharmacokinetics of chemical.

7. For each specific study in which biomarkers are applied, evaluate the population too. Determine if individual variables preclude biomarker use. Do lifestyle, genetic, disease/nutritional variables modify the response?

8. Validate applicability in humans. Conduct a pilot study with a small group of humans with defined exposure gradients to the chemical of interest. Examples of the types of endpoints that may be measured include: cancer, pulmonary, reproductive/developmental.

9. Conduct a demonstration study. Determine that variation in response can be accounted for by exposure and previously identified variables.

10. Apply (validated) biomarker.

Section Four
Future Research Directions

This section identifies the philosophy ORD will follow to develop biomarkers that show promise and should be targeted for further development over the next three to five years (near-term research) and beyond. These tools and techniques will best improve the Agency's ability to evaluate chemical exposures and resultant health effects to support its missions and responsibilities.

4.1 EXPOSURE BIOMARKERS

One of the major areas of concern in biomarker development is the specificity of the tool. Current regulations are chemical specific, and biomarkers are needed to unambiguously identify and characterize exposures to specific chemicals. To meet Agency needs, biomarker research efforts will encompass two types of markers: those that are compound-specific for chemicals of concern (e.g., benzene, trichloroethylene, acrylamide, styrene, nicotine, lead) and those that are indicators of relevant classes of compounds (e.g., dioxins, PCBs). In addition, the program will include three related methods development activities:

1. Evaluation of new scientific information with regard to newly developed biomarkers and biomarker technology, as well as detection method technology (i.e., can they be adapted for Agency use?).
2. Laboratory studies to ensure full characterization of potentially useful biomarkers; refinement/development of pharmacokinetic models for the analysis of biomarker data; and development of monitoring methods and devices for detecting and quantifying biomarkers.
3. Field trials of markers, methods, devices, models, and protocols.

The most promising biomarkers of exposure in the near term are measures related to body burden because they provide measurements of specific chemicals found in the blood, urine, saliva, and breath, thus yielding data directly useable in risk assessment models. Often a breath biomarker is the specific chemical substance under study and the biomarker measurement serves to confirm exposure. Other (non-specific) markers of exposure are useful too because they support assumptions of exposure to a specific chemical or related substances. They include conjugate complexes or elevated or depressed enzyme levels.

Ongoing but longer-term research includes characterization of DNA and protein adducts (including hemoglobin, albumin, and membrane receptor proteins) to detect exposure to toxic chemicals. However, while these measures and others, such as sister chromatid exchange (SCE), may be used as first-tier tests to suggest that exposure to some chemical of concern has occurred, follow-up, chemical-specific tests must then be applied to provide data that are useful for exposure assessment and risk characterization.

For reconstructive exposure assessment to be a practical quantitative tool, a model based on the pharmacokinetics of the particular chemicals in the individual or population of interest is necessary. These models, together with measurements of the specific biomarker, will form the scientific basis to link source, exposure, dose, and health effect.

The following efforts should have the highest priority in future research:

- Interpret/develop biomarkers for body burden (blood and urine sampling), including:

  —Identify and apply markers for field and epidemiological studies on selected subject populations

  —Develop pharmacokinetics models for estimating target tissue dose from surrogate tissue levels and for estimating/reconstructing external exposures from dose measures

  —Identify appropriate metabolites as body burden measures

- Develop and validate new body burden markers for high-priority chemicals (e.g., from Total Exposure Assessment Methodology (TEAM) studies). These markers would likely be:

  —Receptor-xenobiotic complexes

  —Protein adducts

  —DNA adducts

4.2 EFFECTS BIOMARKERS

EPA considers risk versus benefit in the vast majority of its regulatory decisions. The physiological responses that serve as effects biomarkers are not disease endpoints. Unless the relationship between the biomarker and the disease can be shown, the physiologic response will likely be considered too trivial to form the basis for regulatory action. As noted in Section 4.1, most current Agency regulations are chemical specific. Thus, a major emphasis of effects biomarker research at EPA will be to link exposure with health outcome. For each of the priority areas, key needs are outlined below.

4.2.1 Cancer

Most major EPA regulations are based on cancer risk — a framework with profound economic and social implications. Agency cancer risk assessors use a multistage model to quantify the estimated lifetime cancer risk associated with exposure to chemical carcinogens. These assessments currently rely on a number of assumptions (see Section 1.1), and thus may result in either over- or under-regulation of economically useful chemical products. Recent advances in cancer biology, however, are fueling the development of more realistic cancer risk assessment models, which will in turn facilitate the development of regulations that neither underprotect the population nor unnecessarily hinder the industrial use of chemicals.

Two of these advances are the identification of tumor growth, or "onco," genes, and tumor anti-growth, or "suppressor," genes. Studies on these genes in malignant cells of certain tumor types, such as colon cancer, show that the genome of these cells contain more than one genetic change, and suggest that many mutations (perhaps 10-15) must occur before cancer results (Cavanee et al., 1989). Improved knowledge of the mechanism of cancer production following chemical exposure will allow the development of measures for evaluating whether humans have been exposed to carcinogens and for assessing risk. They also provide a basis for intervention to mitigate exposures.

Scientists currently evaluate exposure to carcinogens with DNA and protein adducts because of their chemical or chemical-class specificity. Many uncertainties exist, however, concerning the relationship of these substances to cancer, and many technical difficulties need to be resolved in order to be able to expand promising detection techniques (e.g., the ^{32}P-postlabeling technique) to a wide variety of chemical classes. Initial efforts will focus on the development and application of DNA adducts, stressing:

- Lesion formation
- Rate of repair
- Tissue distribution

and working to:

- Define the dose/response relationship between the adduct and health effect
- Define the relationship between measured adduct levels and target tissue levels if not measured in target tissue
- Determine the lifetime of the adduct
- Establish adduct reference standards
- Extend the methodology to a wide variety of chemical classes

ORD researchers will study protein adducts in order to:

- Relate adducts to target site dose/response
- Develop and improve methodologies and techniques (preparation and identification of adducts)
- Evaluate/refine applicability of biomarkers for field testing

A major emphasis for both DNA and protein adducts will be to:

- Develop adducts for evaluating exposure to complex mixtures (including reference adduct standards) and to identify patterns or arrays of adducts characteristic of chemical classes; these patterns or profiles will be stored on computer files

Regarding the various genes that control cell growth, proliferation, and differentiation, ORD will apply current techniques in molecular carcinogenesis to develop biomarkers for carcinogenesis, including:

- Alterations in DNA:
  - —Mutations in oncogenes and anti-oncogenes
  - —Mutations in DNA repair genes
  - —Chromosomal rearrangements including gene amplification
  - —Multi-locus damage incorporating two-dimensional gel analysis
- Alterations in the expression or structure of proteins or enzymes:
  - —By two-dimensional gel electrophoresis
  - —By Western blots
  - —By radioimmunoassay (RIA) techniques
  - —By immunohistochemistry
- Ultrastructural changes
- Growth factors
- Receptors/receptor-xenobiotic complexes:

—Evaluate the use and interpretation of receptor-xenobiotic complexes for specific chemicals

The risk assessment process for cancer is based on extrapolating human risk from available animal data. The quantified risk factor is generally calculated for the "average" human, even though the human population consists of a heterogeneous mix of diverse genetic backgrounds. This genetic diversity coupled with environmentally induced illness (infectious or chemically induced, such as cigarette smoking) creates a stratified population with varying susceptibility to particular environmental exposures. Biomarkers will be developed to characterize the interindividual variation in the human population and to identify susceptible subpopulations.

4.2.2 Pulmonary

Perhaps the most significant environmental hazard facing citizens of all countries is air pollution, as noted by EPA in the Unfinished Business Report (EPA, 1987). Automobile emissions and industrial outgassing result in significant human exposure to ozone, nitrous oxides, sulfur oxides, and carbon monoxide. In addition, household furnishings, cleaners, and lifestyle habits (e.g., cooking, smoking, dry cleaning) can result in significant exposure to many other substances.

The lung is the primary route of entry for air pollutants. Most of the regulations promulgated under the Clean Air Act are based on data showing effects to the cardiopulmonary system. Because of the lung's relative accessibility, EPA and others have extensively used biomarkers from lung fluids and tissue to assess human exposure to air pollutants. Most of these studies have been conducted in a clinical setting, often with invasive techniques. A major goal of research on pulmonary effects biomarkers is to develop, validate, and apply less invasive markers on larger populations than is now possible.

The following pulmonary biomarkers have the highest priority for future research:

Near term:

- Develop methods for assessing the effects from, and exposure to, acid aerosols
- Develop new markers for use with urine and blood
- Develop markers for ozone degradation products (DNA, protein products)
- Strengthen interpretation of nasal lavage (NAL) biomarkers
- Strengthen interpretation of biochemical/cellular markers
- Strengthen interpretation of molecular biomarkers

Longer term:

- Develop biomarkers for susceptibility (responders versus nonresponders)

4.2.3 Neurotoxicity

A recent Office of Technology and Assessment Report (U.S. Congress, 1990) has identified neurotoxicity as a major health outcome following exposure to environmental pollutants. Many EPA regulations are based in whole or in part on measures of neurotoxicity (e.g., from organophosphate pesticides, metals in drinking water, lead in gasoline). Many of these measures, however, are invasive or assess nonreversible effects following exposure. ORD's research in neurotoxicological effects biomarkers focuses on developing relationships between exposure and outcome for cholinesterase and neurotoxic esterase and developing minimally invasive techniques to detect nervous-system-specific proteins that point to the early steps of neurotoxicity.

Research efforts will thus be designed to:

- Determine the relationship between cholinesterase activity in blood, peripheral nervous system, and central nervous system.
- Determine the relationship between the degree of cholinesterase inhibition, neurotoxic esterase inhibition, and neurotoxic outcome.
- Determine whether age and/or species differences exist in the above relationship.

Other longer-term priority biomarkers under development include:

- Gel electrophoretic protein profiles from cerebrospinal fluid (CSF)
- Presence of nervous system-specific proteins and degradation products in CSF, plasma and urine
- Presence of immunoglobulins in serum directed at brain-derived antigens
- Presence of neurotransmitter metabolites in CSF
- Monitoring applications in blood and urine
- Subcellular antigens in blood and urine pointing to the extent and localization of damage to the nervous and other organ systems

4.2.4 Reproductive/Developmental

Humans experience a significant reproductive failure rate. Exposure to environmental chemicals has been associated with infertility, and EPA regulates a number of chemicals (e.g., dibromochloropropane, nitrofen, dinoseb) thought to cause this problem. In developing and using biomarkers to

support the Agency's regulatory activities, ORD researchers will focus their efforts first on males, due to the relative ease of obtaining samples from men of reproductive age. As they become available, however, techniques will also be used for better assessing female infertility.

The following reproductive/developmental biomarkers will have the highest priority for future research:

Near term, male:

- Improve *in vitro* tests of sperm function
- Improve predictive value of semen analysis; also included are efforts to improve sampling techniques, standardize results, and develop suitable sample containers
- Validate measurement of testosterone in saliva
- Develop methods to assess the genetic integrity of sperm (e.g., DNA damage, chromatid structure) and generalized screening methods for genetic integrity

Near term, female:

- Multiple sputum samples for steroids to validate the sensitivity of the approach
- Validate measurement of LH (ovarian cycle protocol) in mid-cycle urine

Near term, male and female:

- Develop noninvasive techniques for human reproductive toxicology based on animal models
- Identify better markers indicative of early changes in reproductive function

Longer term, female:

- Develop noninvasive methods for biomarkers of preimplantation development

Longer term, male:

- Develop better markers of sperm membrane integrity and *in vitro* tests of sperm function

The highest priority for general research will be given to efforts to:

- Develop and validate a questionnaire and/or decision tree to identify and assess fertility problems in the male and female specific to EPA's needs; existing protocols will be standardized
- Develop and validate noninvasive analytical markers from urine and saliva that would allow more frequent measures of endocrine control of reproductive function
- Develop and validate biomarkers to monitor early pregnancy and to distinguish between pre- and post-implantation loss
- Improve the predictive value of semen analysis, including improved methods of multivariant analysis and further identification of molecular and biochemical markers of sperm function

4.2.5 Immunotoxicity

Many *in vivo* and *in vitro* tests can be used to evaluate immune system responses in humans. Scientists have used these tests to demonstrate allergic reactions to environmental chemicals and altered immune functioning following exposure to environmental pollutants. However, because of the complexity and interactive dynamics associated with immune responses, it has been difficult to interpret these responses for their adverse health impact on humans. Consequently, with the exception of allergic responses, risk assessors have had difficulty using immune system responses as a basis for regulatory decision-making because these responses are not severe enough to be considered a significant adverse health impact. ORD research in immunotoxicity will focus on interpreting the significance of immune response parameters and on using immune responses to link exposure to effects (in both immune and other target organs).

The following immunotoxicity biomarkers will have the highest priority for future research:

- Standardize procedures and techniques
- Improve biomarker data interpretation; consider such improvements for their impact on the development and prioritization of other biomarkers
- Improve the ability to extrapolate from animals to humans and determine species relevance for human hazard identification
- Develop sensitive immunological markers (with identifiable responses at threshold levels)
- Develop more quantitative markers of exposure for dose determination (long term and low probability of success, but potentially high payoff)
- Improve the interpretability of biomarkers of immunotoxicity in order to predict susceptibility to disease
- Develop and validate biomarkers that are indicators of hypersensitivity responses
- Improve the understanding of the mechanism(s) of chemical-induced immune alterations (i.e., immunosuppression, hypersensitivity and autoimmunity) and the identification of biomarkers for such chemicals

4.2.6 Heritable Genetic Mutations

Heritable mutation research will utilize new recombinant DNA techniques to evaluate mechanisms of mutation in germ cells. The significance of genetic recombination and segregation events at meiosis leading to mutation will be analyzed to achieve a better understanding of germ-line specific targets and mechanisms in the induction of heritable mutations, in order to identify appropriate biomarkers. The greatest challenge will be to develop noninvasive techniques for use on human subjects to identify mutant phenotypes. In addition, the research projects will be undertaken to:

- Evaluate the scientific literature and newly emerging epidemiology studies for evidence of environmentally induced heritable damage in humans
- Evaluate and develop rodent models for induced heritable damage to identify new biomarkers
- Utilize new molecular and cytogenetic techniques for monitoring offspring for newly induced genetic damage in humans

4.2.7 Hepatotoxicity

Because of the variety of serum markers of hepatic damage available and the varying specificity and sensitivity of these markers, near-term research will focus on defining an optimal battery of serum biomarkers suitable for routine environmental monitoring. Results of interlaboratory proficiency studies indicate marked variability in the results of serum enzyme tests (Blanchaert, 1987). Research efforts will determine the causes of this variability and suggest methods to reduce its effect on the outcome of studies. Research is also needed on noninvasive tests for hepatic fibrosis and cirrhosis; examples of research areas include serum/urine measurement of proline and hydroxyproline and serum assay (immunoassay) of type III procollagen.

Epidemiologic studies of populations exposed to toxic waste sites are limited by the technical and human problems outlined by Marsh and Caplan (1986), including small population size, heterogeneity of the exposed population with respect to characteristics that can influence health outcome, poorly defined exposures, and health outcomes that are rare or have long latency periods. The ORD program will examine the influence of these variables on the outcome of biomarker monitoring studies.

In addition, priority will be given to efforts to:

- Evaluate/develop noninvasive clearance techniques; examples of research areas include evaluation of usefulness of spectrophotometric measurements of dye clearance in ear veins for human population monitoring; aminopyrine clearance
- Validate/develop markers for changes in hepatic cytochrome P-450 content and composition and hepatic glutathione content; research will interpret results, determine delivered dose/effect relationships, and define the chemical specificity of the methods
- Validate/develop serum markers to determine the location of damage in liver acinus/lobule and subcellular location of damage

4.2.8 Other Organ Systems

For other organ systems, future research efforts will focus on:

- Kidney biomarkers
- Cardiovascular biomarkers targeting morbidity/mortality factors
- Biomarkers for organ-specific gene expression

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