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EPA

Research to Improve Health Risk Assessments (RIHRA) Program

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Office of Research and Development
U.S. Environmental Protection Agency
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FOREWORD

To realistically assess the human health risks associated with exposures to environmental pollutants, we must understand a number of important physical, chemical, and biological processes and mechanisms. This suggests that real progress in enhancing the accuracy and precision of health risk assessments depends on the products of a research program focused on the underlying mechanisms of exposure, dose, and effects. Although much of the U.S. Environmental Protection Agency's (EPA's) base research program has been targeted historically on these issues, substantial benefits can still be gained from instituting a new, more focused research program aimed specifically at reducing the critical uncertainties associated with health risk assessments.

The goal of this program, known formally as the Research to Improve Health Risk Assessments (RIHRA) Program, is to generate research results that will significantly improve the EPA's ability to assess human health risks. The RIHRA program is structured so that the research products are germane to a cross-section of the Agency's regulatory programs and risk assessment needs. We are confident that the mission-oriented research described in this document will provide the EPA with credible scientific findings that are relevant for risk assessment decisions.

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Director, Office of Health Research
Chairman, RIHRA Committee

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EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) must assess the health risks of environmental exposures in order to make regulatory decisions that safeguard public health. The Agency is limited in its ability to assess health risks quantitatively because of a lack of understanding about the underlying biological, chemical, and physical processes that determine exposures and effects. Without sufficient knowledge of these processes, uncertainties are introduced into the risk assessment process that allow wide interpretation of the limited available data. Thus, the current database may support diametrically opposed assessments of risk, each with dramatically different ramifications for related regulatory decisions.

Targeted, long-term research is needed to reduce these uncertainties in health risk assessment. To meet this need, EPA's Office of Research and Development (ORD) established a systematic and integrated Research to Improve Health Risk Assessments (RIHRA) program. This research program is designed to provide critical data on the relationship between exposure, dose to target tissue (delivered dose), and associated health effects. The program emphasizes laboratory and field research to improve understanding of basic biological mechanisms, especially as they relate to our ability to extrapolate from one set of circumstances (e.g., animals exposed to short-term, high concentrations) to another (e.g., humans exposed to long-term, low concentrations). In implementing an integrated and systematic research effort, the RIHRA program builds on existing capabilities within ORD and ongoing intramural efforts. Data gathered in projects under the RIHRA program will enhance our ability to quantify the human health risks associated with environmental exposures.

INTRODUCTION

BACKGROUND

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, however, EPA currently relies on quantitative assessments of the health risks associated with different pollutants. 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.

In 1988, Congress recommended that EPA's Office of Research and Development (ORD) establish a systematic and integrated Research to Improve Health Risk Assessments (RIHRA) program. Although much of ORD's ongoing research focuses on this issue, the Agency saw benefit in a more formal, structured approach. Consequently, $3 million in fiscal year (FY) 88 and $10 million in FY89 ($7 million for health; $3 million for ecology) were earmarked for development of a targeted, coherent research program to reduce the uncertainties in the risk assessment process.

COMPLETE RISK MODEL

To understand the human health risk associated with environmental exposures, the principal relationships between the various sources of a pollutant and the pollutant's effects on a target population must be defined. Establishing these connections for a particular target and pollutant is a critical task in any effort to reduce total risk from that pollutant. We can construct the causal chain joining a pollutant source to effect—and understand the biological, chemical, and physical processes that underlie human exposure and response to the pollutant—through an evaluation of each of the four components of, or links in, the chain:

- Source of the pollutant
- Movement of the pollutant from the source to the target resulting in exposure of the target to the pollutant (NOTE: RIHRA is concerned primarily with the human population as target; a companion program on ecological risk assessment is in preparation)
- Dose received by the target caused by this exposure
- Adverse effects resulting from the dose

The informational output for one component in the chain is the input for the next. A mathematical formulation representing this entire chain would constitute a complete risk model for the pollutant.

RISK ASSESSMENT FRAMEWORK

Assessing the risk associated with exposure to a given pollutant requires a sufficient understanding of each of the four links in the complete risk model. If the necessary information for a single link is missing, the chain between source and effect cannot be fully characterized. It is possible to focus on a single link in the chain in developing mitigation strategies—for example, strategies aimed at the sources or movement patterns of a pollutant or the behavior of a target may effectively mitigate risk. Estimates of the residual health risk, however, must still be derived after implementation of such strategies.

The steps in risk assessment mirror the links in the chain joining the human population to pollutant source(s). Given the inadequacy of current databases, these steps also represent key issues in environmental health research:

- **Exposure Assessment.** What environmental exposures occur or are anticipated to occur for relevant human populations?
- **Hazard Identification/Dose-Response Assessment.** Does the agent cause an adverse health effect? What is the site(s) of toxic action within the body and what is the pollutant dose delivered to that site(s)?

- **Risk Characterization.** What are the relationships among exposure, target dose, and adverse health consequences?

UNCERTAINTIES IN RISK ASSESSMENT

The causal pathway between pollutant source and heath effect cannot be fully characterized for every possible environmental scenario. It is not possible to fully research every set of exposure conditions in relation to every possible pollutant, or to examine the particular susceptibility of every human subgroup to effects from a pollutant. In addition, much data on response to pollutants must be gathered from laboratory animals under an entirely different set of exposure conditions than humans experience. The most critical problem in risk assessment—how to make accurate extrapolations—develops from these limitations. An unacceptable level of uncertainty is currently associated with efforts to extrapolate observed effects from one set of circumstances (e.g., cancer incidence in rats subjected to high, chronic exposures in controlled experiments) to an entirely different set of circumstances (e.g., individual excess cancer risks in humans experiencing intermittent, low-level exposures).

Researchers encounter these uncertainties while extrapolating from species to species, from one individual or subgroup to another individual or subgroup within a particular species, or from one set of exposure conditions to another. The uncertainties spring not only from the insufficiency of data, but also from a lack of fundamental understanding about the relevant underlying physical (e.g., atmospheric dispersion characteristics, human activity patterns), chemical (e.g., chemical reactions and transformations), and biological (e.g., metabolism, disease processes) mechanisms that affect the validity of the extrapolation assumptions.

For example, most risk assessments in the past have assumed by default that an equivalency of response exists between animals and humans. As one proceeds from the molecular level or biochemical event toward injury at the tissue or organ level, that assumption may become less tenable. A sufficiently high dose can produce damage at any level from the target molecule to the intact organ, resulting in various disease states. On the other hand, host defense and/or compensatory systems could intervene to prevent or repair the damage. Disease outcome is also heavily dependent on the size of the delivered dose and dose-time relationships. The lack of information on these related events results in significant uncertainty in current risk assessments and, as a result, the use of a variety of default assumptions.

Under RIHRA, careful consideration of the assumptions made at various stages of the risk assessment process has highlighted areas in which research can improve risk assessments by providing scientifically defensible data to replace these assumptions. Even if the assumptions cannot be refuted, the data may support more defensible defaults. In either case, such research will increase our confidence in making health risk assessments.

DEVELOPMENT OF THE RIHRA PROGRAM

Selection of Topics

The RIHRA program is designed to focus limited resources into a structured and integrated program for reducing uncertainties in the risk assessment process, thus enhancing our ability to quantify the human health risks associated with environmental exposures. ORD has taken three significant steps to focus the program:

1. Collective judgment and understanding of the risk assessment process was used to narrow the scope of the program. Accordingly, the RIHRA program will focus on elucidating the relationship between exposure, internal dose to the target tissue, and associated health effects. The RIHRA program does not encompass efforts to measure ambient concentrations of pollutants for exposure assessments or to characterize sources; instead, it studies concentrations of pollutants at the point of human contact as well as the health effects associated with that contact. Nor does it encompass efforts in hazard identification—which is an area of research covered by the ORD base program (see Program Implementation).

2. Special emphasis will be given to noncancer health endpoints, such as neurological, pulmonary, and reproductive effects. This emphasis is appropriate because EPA is increasingly called on to estimate these kinds of risks for environmental exposures to a variety of pollutants. In general, the risk assessment issues are less well-defined and articulated for noncancer than for cancer effects.

3. The program will involve extensive short-term and medium- to long-term research planning to achieve the necessary stability. Stability is

required because research to reduce the uncertainties of risk assessment is iterative in nature; that is, the research progresses in multiple stages, with significant outputs at each stage.

In light of this focusing effort, ORD chose four major topics for inclusion in the program (the relationship between these topics and factors in the risk assessment process can be seen in Figure 1-1):

- **Topic 1: Analysis of Uncertainty in Risk Assessment.** The degree of uncertainty associated with the qualitative and quantitative aspects of risk assessment is often poorly understood. Research related to this topic addresses clarifying and quantifying the uncertainty associated with each assumption.

- **Topic 2: Integrated Exposure Assessment.** Exposure assessment (i.e., contact between chemicals and humans) is based on either ambient or

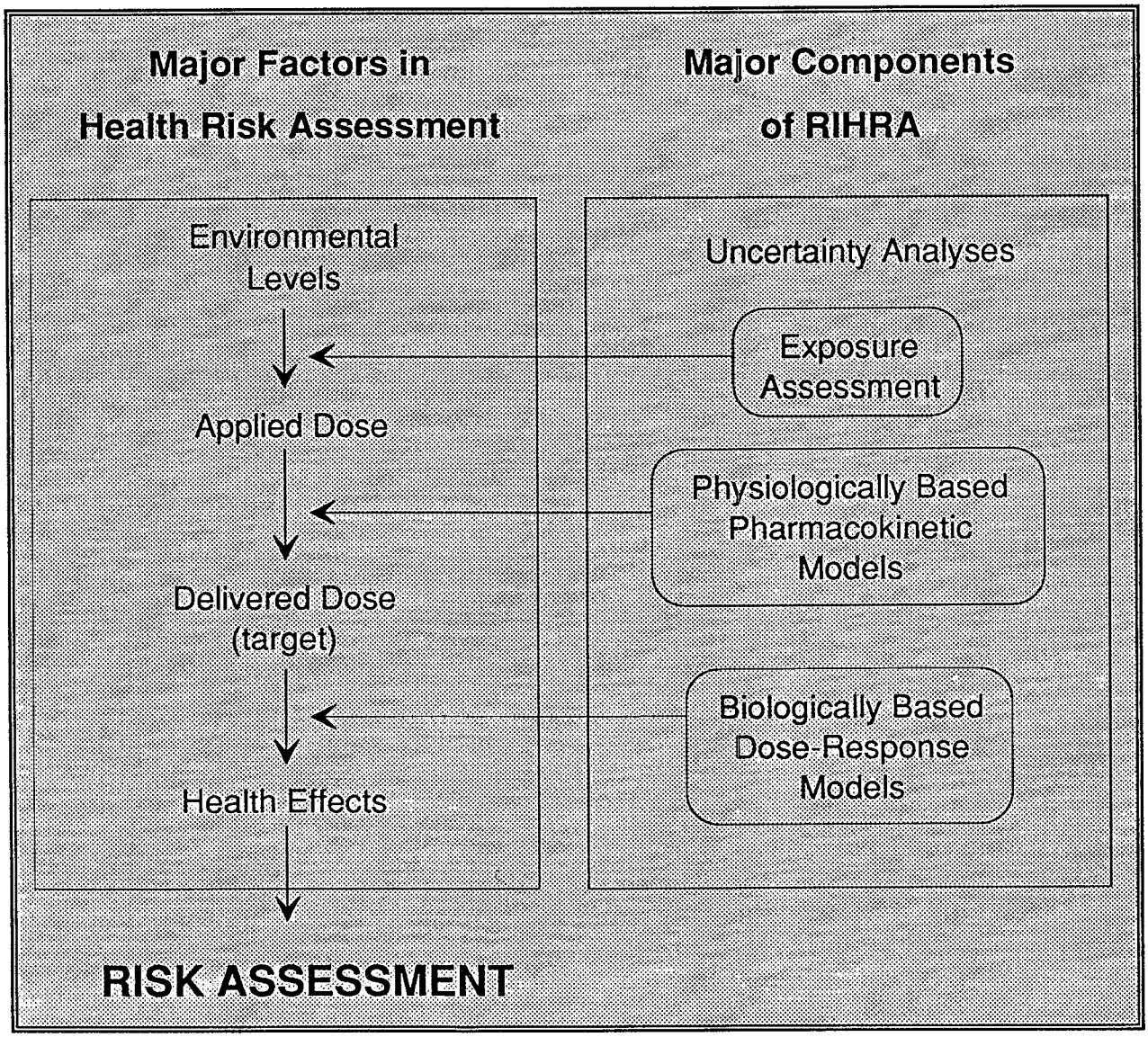

Figure 1-1: Relationship between the major factors in health risk assessment and the major components of the RIHRA Program.

biological measurement. Ambient measurements can be further subdivided into direct (e.g., individual monitoring) and indirect (combining human activity data with pollutant measurements in important microenvironments) approaches. Improvements are needed in the quality and consistency of data used to assess exposure with either approach.

- **Topic 3: Physiologically Based Pharmacokinetic (PB-PK) Models.** The concentration of a pollutant to which a human is exposed is usually not the same as the concentration of pollutant delivered to the target organ (the dose). A number of mechanisms, many poorly understood, affect the transport of the pollutant through the portal of entry (e.g., the lung for an inhaled pollutant) to the target organ(s). In risk assessments, however, the exposure concentration of a pollutant is often used as a surrogate for the dose concentration because the data on the latter are not available. Better dose data will help reduce the uncertainties associated with extrapolating effects from one route of exposure to another, from chronic to acute exposure, from high to low exposure, and from one species to another. While biological factors must also be considered when assessing risk, first scientists must account for the effects of the duration, magnitude, and frequency of the exposure on the dose to the target organ (i.e., pharmacokinetics). PB-PK models can be used to establish a quantitative relationship between exposure and dose delivered to a target site in animals and humans under a variety of conditions.

- **Topic 4: Biologically Based Dose-Response (BB-DR) Models.** A key element in the risk assessment process is to estimate the incidence of a specific health effect in human subpopulations. Because human exposure data are usually not available, scientists must extrapolate the risk of health effects from data gathered with laboratory animals. Research under this topic will be heavily oriented to mechanistic studies aimed at facilitating more biologically valid extrapolations from animal data, and thus reducing uncertainties in this step of the risk assessment process. The development of accurate BB-DR models will allow scientists to better define a quantitative relationship between the dose of a pollutant and associated health effects in humans.

Selection of Projects

To determine whether projects were appropriate for inclusion in the RIHRA program, ORD applied the following four criteria in the selection process:

1. *Does the project focus on major significant uncertainties and knowledge gaps in Agency risk assessments?* Important areas for research include assumptions and extrapolations that are used frequently and in which we have little confidence.

2. *Does the project have a reasonable probability of success?* The research must be technically feasible in terms of existing expertise, resources, and knowledge.

3. *Would results of the research directly support the needs of the Agency's risk assessors?* Scientists in EPA and elsewhere should be able to apply the results of the research in risk assessments. In aggregate, the projects were chosen to produce a mixture of short- and long-term products for use by the Agency in risk assessments.

4. *Will the project data be amenable to wide application?* Results should be useful in a broad context rather than limited to specific situations. Ideally, the short-term products of this research can be immediately applied to Agency needs and can also be used as the logical building blocks for long-term improvement of risk assessment methodologies.

Furthermore, the projects chosen had to be consistent with the EPA mission and represent the kind of work that EPA and ORD are expected to perform; that is, the projects must provide results that are appropriate to EPA's legislative mandates and regulatory authorities, and are useful to Program Offices. These same criteria will be applied to the choice of RIHRA projects in the future.

Program Implementation

The EPA Multimedia Research Committee—and, more specifically, the RIHRA Subcommittee—has formal responsibility for the RIHRA program. The subcommittee, which is composed of the Office Directors (and other key designees) from the participating ORD Offices (see Figure 1-2), designs, implements, and manages the program. In addition, the subcommittee has two other tasks. First, it coordinates the intramural (e.g., Program Offices, Science Advisory Board, Risk Assessment Council) and

extramural (e.g., outside peer-review groups) reviews of the RIHRA program, and ensures that the reviews are used to strengthen and improve the research program. Second, it has established working groups for each of the major RIHRA research topics, composed of one permanent member from each participating Office and a varying number of other ORD scientists. The working groups integrate input from their respective Offices to develop a focused research plan (down to the project level) for each topic. These research plans are reviewed and approved by the subcommittee.

Implementation of the RIHRA research plans under each topic highlights the following characteristics of the program:

- **RIHRA complements the ORD base research program.** The ORD base research program is funded primarily through ORD Research Committees for each medium (e.g., air, water). These committees, which are composed of ORD and Program Office personnel, primarily support targeted research on risk assessment issues or chemical-specific topics to meet immediate needs of Program Offices. The RIHRA program also supports short-term research, but it concentrates on longer-term efforts that may be augmented by base program capabilities.

- **RIHRA comprises many cross-media projects.** The RIHRA program categorizes projects in a hierarchy: projects fall under issues and issues fall under topics. RIHRA research activities, however, often contribute important data to efforts under more than one topic (e.g., PB-PK modeling as well as exposure assessment) and to examinations of more than one medium. In contrast, most projects developed under the ORD base program are designed to answer needs of a specific Program Office—and thus focus on a single medium.

ORGANIZATION OF DOCUMENT

This document summarizes the research that is both under way and proposed for the RIHRA program. This summary is organized by the fundamental topics chosen for the program: analysis of uncertainty in risk assessment (Section 1), integrated exposure

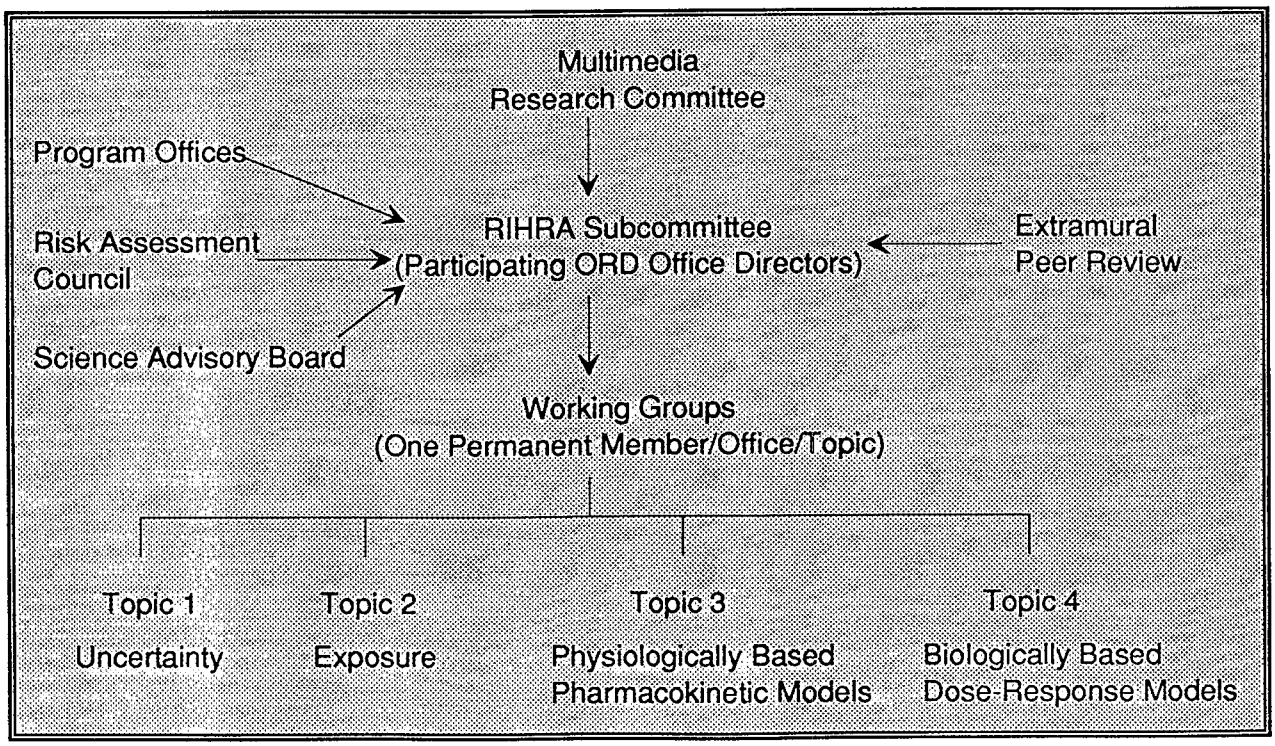

Figure 1-2: Proposed implementation scheme for the Research to Improve Health Risk Assessments (RIHRA) program.

assessment (Section 2), physiologically based pharmacokinetic models (Section 3), and biologically based dose-response models (Section 4). Each section provides further detail and a research strategy for work under that topic. Project descriptions are included in the Appendix for each topic. Note that program overviews for work in different fields (e.g., neurotoxicology, developmental toxicology) under Topic 4 are provided in Appendix D.

TOPIC 1
ANALYSES OF UNCERTAINTY IN RISK ASSESSMENT

1.1 ISSUES COVERED

The importance of understanding uncertainty in the estimates of risk produced by Agency risk assessors cannot be overstated. Without such an understanding, risk assessors—and for that matter, risk managers—fall prey to the "tyranny of numbers" that can affect their ability to put risk characterization, both qualitative and quantitative, in proper perspective. Such lack of perspective can lead to inappropriate comparisons of risk across chemicals and can result in faulty decision-making with regard to chemical-specific issues.

The issue of uncertainty analysis cuts across all of the RIHRA topics; but for Topic 1, the specific goal is the development of a research and data collection strategy to deal with generic aspects of the issue. A framework is needed to delineate the sources and magnitudes of uncertainty associated with Agency risk assessments, and to explore various approaches for describing or reducing these uncertainties. Within this framework, researchers will examine whether a particular uncertainty is of sufficient magnitude to significantly impact regulatory decisions. If it is, the type of uncertainty (e.g., extrapolation, source assessment) will be described and, if possible, quantified. Efforts will also include investigation of what the end users of risk assessments (i.e., decision makers) need with respect to assessment of these uncertainties, both quantitatively and qualitatively. As a result of this work, researchers will be able to identify the sources of uncertainty in risk assessment and to explore various approaches to reducing these uncertainties.

Projects under this topic might include exploration of possible approaches to analyzing uncertainty that build on such formal disciplines as decision analysis theory or Monte Carlo simulation. A number of approaches have been recognized as potentially useful, but further work is needed to select the most appropriate ones.

1.2 DEVELOPMENT OF A FRAMEWORK

In view of the breadth and depth of Topic 1, a scoping workshop of active investigators in the area of uncertainty analysis was set up to develop a strategic framework; the group was also given the task of identifying projects for potential funding in FY89 and FY90. The workshop, which took the form of a "Seminar on the Use of Analytical Methods for Uncertainty and Sensitivity Analyses in Pharmacokinetic Studies and Risk Assessments," was held at the Dulles Marriot Hotel in Virginia on December 7 and 8, 1988. Participants were informed of the Agency's intent to develop a framework for evaluating uncertainties in risk assessments, and to identify innovative research aimed at describing and/or reducing uncertainties. Presentations by ten invited participants were followed by a discussion session of interested Agency participants.

A number of approaches to an overall framework for classifying uncertainties were presented and discussed. The consensus opinion preferred a scheme presented by Max Henrion of Carnegie Mellon University, which has now been adopted as a means of stratifying uncertainties and categorizing research proposals in this issue area. The scheme is based on sources of uncertainty, as listed here:

1. Random error/statistical variation
2. Systematic error/subjective expert judgment
3. Disagreement between experts
4. Linguistic imprecision
5. Interindividual variability
6. Model approximations/model simplifications
7. Model structure/fundamental assumptions

In addition to discussing framework development, Agency participants provided feed-back on specific research proposed by the invited experts. This process has resulted in the research strategy described in the Section 1.3.

The elements of this framework allow for a comprehensive evaluation of uncertainties in risk assessments. In addition, each of these elements can be evaluated with regard to available or proposed research to address uncertainties that fall within it. This approach leads naturally into the development of a research strategy.

1.3 RESEARCH STRATEGY

To develop a research strategy for uncertainty analysis within the limited resources available under Topic 1, ORD took into account ongoing work in the

base program (see Introduction) and assessed which projects would be best accomplished under other RIHRA topics. Workshop participants preferred a concentration on the development of structured presentations of issues in uncertainty analysis and on the elements of the framework related to use and evaluation of expert judgment (#2 and #3), linguistic imprecision (#4), the influence of interindividual variability on uncertainty (#5), and implications of model approximations/simplifications (#6). ORD determined that work on random error/statistical variation (#1) and on fundamental model structure (#7) were more properly the purview of Topics 2, 3, and 4 of the RIHRA program. These latter elements of uncertainty are integral parts of data collection and model validation, which are major components of the research efforts under the other topics. Several projects presented by experts at the workshop were compatible with the expressed emphasis of this strategy; others were judged not to be as pertinent. From the ten proposals made at the workshop, those pursued for FY89 are presented in Table 1-1. A detailed description of Topic 1 projects is included in Appendix A.

TABLE 1-1

PROJECTS FUNDED UNDER TOPIC 1
ANALYSES OF UNCERTAINTY IN RISK ASSESSMENT

TOPIC/ ISSUE	PROJECT OFFICER	SHORT TITLE	STATUS	FY89($K)	DURATION
1.2	Farland	Review of "Guidelines"	Coop* - National Academy of Sciences	35	FY 89
	Farland	Addressing Uncertainties for Exposure Model	Coop - Lawrence Livermore National Lab	90	FY 89
	Farland	Interindividual Variability and Uncertainty	Coop - Lawrence Livermore National Lab	90	FY 89
	Farland	Evaluation of Communication	Coop - Harvard	35	FY 89

*Coop = Cooperative Agreement

TOPIC 2
INTEGRATED EXPOSURE ASSESSMENT

2.1 ISSUES COVERED

Information about the number of people exposed to pollutant(s) and their degree of exposure is an essential component of any risk assessment. Estimating human exposure, however, has proved difficult and may be the single largest source of uncertainty in environmental risk assessments.

Over the past several years, researchers at EPA and elsewhere have assessed exposure and dose to the target organ(s) in three ways, one direct and two indirect. Direct techniques require real-time measurements of contact intensity by personal monitors, such as radiation badges, active devices that pump and trap volatile chemicals, or chemical analysis of the amounts and contamination levels of food and water ingested. Indirect measurement can either be *predictive*, using models for pollutant behavior and/or human (ecological) behavior; or *dosimetric* (under limited conditions), using body burden and knowledge of pharmacokinetics to back-calculate the exposure from observed levels in the body. All of these methods are limited by associated uncertainties. Dosimetry is discussed under Topic 3.

Predictive exposure assessment techniques are appealing because, by using them, regulators can evaluate the impacts of regulatory options on risk. Exposure models can not only estimate concentrations of pollutants in the various media, but can also link particular environmental concentrations to exposure levels in target populations with different activity patterns. Such models are needed because humans are mobile in the environment, and thus assuming a constant level of exposure over time for an individual or population is at best an approximation, and at worst a major misrepresentation of real-time exposure. In the past, insufficient data on human activities and activity patterns and a lack of methods to incorporate this information have been weak links in risk assessment—and thus sources of great uncertainty. The first two issues discussed under this topic (2.1 and 2.2) therefore deal directly with reducing that uncertainty through development of models that can accurately predict exposure, taking human activity patterns into account.

Measurements of source contributions to pollutant levels have also been too limited. Pollutants have customarily been measured only in carrier media (e.g., outdoor air, streams, soil, or food). This approach relies on the assumption that limiting pollutants in important carrier media will bring about the desired protection of public health and welfare—for example, that efforts should concentrate on reducing outdoor sources of pollution to the point that observed outdoor air quality meets national standards (Clean Air Act).

RIHRA exposure models will support an alternative approach, termed **risk-based environmental management**, in which <u>all</u> sources that contribute to exposure and risk are evaluated. In other words, carrier media of long-standing concern (e.g., outdoor air, drinking water) and sources of pollutants operating through these media are included in the strategy, but so are building materials (e.g., adhesives, carpets, floors), consumer products (e.g., furniture, sorbents, pesticides), and other sources of pollution (e.g., soils and dust, vehicular traffic) if these sources contribute to the total risk from a pollutant. Implementing risk-based environmental management involves:

- Defining whose risks are going to be reduced; the **target**, or receptor, of the risks could be a human population, an animal species, or an ecological system
- Determining the causes of the risk
- Reducing the risks to acceptable levels by controlling the causes of the risk in a manner suited to their relative importance; for example, if source A of a pollutant contributes 95% of the risk and source B only 5%, then most control efforts should concentrate on source A

This integrated exposure assessment approach leads to improved efficiencies in reducing risk because it focuses resources on the sources of the greatest amount of risk. Also, allocating risk reduction efforts by the relative contribution of each source to total risk and assessing the cost to society of each component of the risk reduction effort can increase cost-effectiveness.

Another major source of uncertainty covered under this topic is the means by which exposure data are gathered in the field. For risk assessors to reach conclusions with minimal uncertainty, data-gathering methods must be appropriate to the use of the data, whether in predictive assessments or direct measurements of exposure. For example, how do a series of short-term duration exposure peaks differ in a risk

context from lifetime long-term exposures at fairly constant but lower levels? And how can this information be best measured in the field and incorporated into the exposure assessment? Efforts under Issue 2.3 address this concern, and are related to work proposed under Issue 4.3 (which addresses how information from different exposure conditions would be used in a dose-response relationship). Taken together, results from projects under Issues 2.3 and 4.3 will reduce uncertainty in risk assessments by making the assessments more closely describe real events.

A final major uncertainty source associated with exposure estimation is the inconsistency in assumptions, methodology, and parameter values used by various assessors for similar exposure situations. This variation often leads to substantially different estimates of exposure or risk for the same situation (for example, from EPA and a company sponsored by a potentially responsible party on a Superfund site). Work under Issues 2.1 and 2.3 is improving EPA's approach to exposure assessment by gathering data that can be used consistently across many assessments and by standardizing the approach to estimations of population exposures for many commonly evaluated situations (e.g., incinerators, waste sites, indoor air).

Issue 2.1: Human Exposure Models

ORD will develop and validate human exposure models designed to generate realistic predictions of exposure to chemicals using data on human activity patterns and exposure sources. This type of model links the concentrations of pollutants to which people are exposed in various microenvironments (i.e., microenvironmental exposures) with the time spent in those microenvironments (human activity patterns), and integrates this information with the pollutant doses associated with the length and intensity of the exposure. Human exposure models should address all the microenvironments in which people visit or reside (e.g., homes, stores, places of religious worship, schools, buses, automobiles, workplaces), and must take into account multiple possible routes of exposure (air, food, drinking water). Indoor and in-transit microenvironments are particularly important. Research in this area will include developing these models using existing data (2.1.1), and also validating these models (2.1.2).

2.1.1: Development of Human Exposure Models

Research in this area will focus on constructing human exposure models for important pollutants that EPA regulates (e.g., respirable particles, volatile organic compounds, semivolatile organics, formaldehyde) using the best microenvironmental data and the best activity pattern data available. If microenvironmental data are unavailable, researchers will conduct special field investigations to construct the needed submodels. The human activity pattern and microenvironmental concentration values will combine human activity, time budget (see Issue 2.2), and microenvironmental concentration data using a generalized exposure equation. Researchers will incorporate suitable pharmacokinetic models into the exposure models to estimate body burden and dose for the regulated pollutants.

2.1.2: Model Validation

The human exposure models will be validated by comparison of the resulting predictions to field data collected in total human exposure field studies for a variety of situations. Uncertainty ranges around the predictions will be characterized. Then, teams will develop guidance concerning the appropriateness and the accuracy/uncertainty of the models for various exposure situations.

Issue 2.2: Human Activity Patterns

Research efforts will characterize human activity patterns in order to improve exposure analyses in risk assessments. Human activity patterns, sometimes called "time budgets," are records of what people do, where, when, and for how long. Recent field studies of human exposure, such as the Total Exposure Assessment Methodology (TEAM) studies, have shown that an individual's activities are critical in determining exposure to environmental pollutants.

Once sufficient human activity data have been gathered, the Agency will be able to realistically predict individual exposures through the use of probabilities. Significantly, the Agency will no longer have to rely solely on a worst-case scenario in predicting a population exposure. Instead, with activity pattern information, risk assessors can develop a frequency distribution for exposure, and thereby support predictions concerning "average" exposure, exposure in the 90th percentile, and so on.

Issue 2.3: Database on Indirect Exposure Parameters

Researchers will expand the database on the parameters used to make indirect exposure estimates and will clarify how to use these estimates. More information is needed on the ranges and distributions of parameters used in indirect exposure assessments,

such as ingestion rates, exposure durations, contact rates, and short-term versus long-term exposures. The Regions have repeatedly requested guidance on such topics to improve consistency in risk assessment. Guidance is also needed on ways to apply these factors in creating different scenarios, such as a typical and a worst-case exposure level.

Two concerns that will receive special attention under this issue relate to 1) predicting the incidental soil ingestion of children based on site-specific parameters such as ground cover and weather, and 2) determining the contribution of short-term peaks to total exposure. Soil ingestion by children, an activity that has stirred significant controversy regarding its importance, is an important exposure route in many risk assessments. For many pollutants, short-term peaks are extraordinarily important contributors to exposure. Besides their contribution to exposure, short-term peaks affect the dose-response portion of risk assessment (see Issue 4.3). Teams will pursue methods to measure short-term peaks and then use this information to establish dose-response relationships.

2.2 GATHERING EXPOSURE DATA

The ORD base exposure program (see Introduction) has emphasized these research areas:

- Developing and evaluating methods for conducting total human exposure studies for several pollutants or pollutant classes (e.g., volatile organic compounds [VOCs])
- Developing instrumentation

These two efforts together form the scientific basis from which to develop the exposure model framework (Figure 2-1). Embodied within this framework is the monitoring systems approach, which includes monitoring and statistical design, quality assurance objectives, information requirements, Office of Management and Budget (OMB) clearance, and information assessment.

To fill the critical information gaps that remain in exposure assessment, further efforts in both direct and indirect measurements must be made. Only with

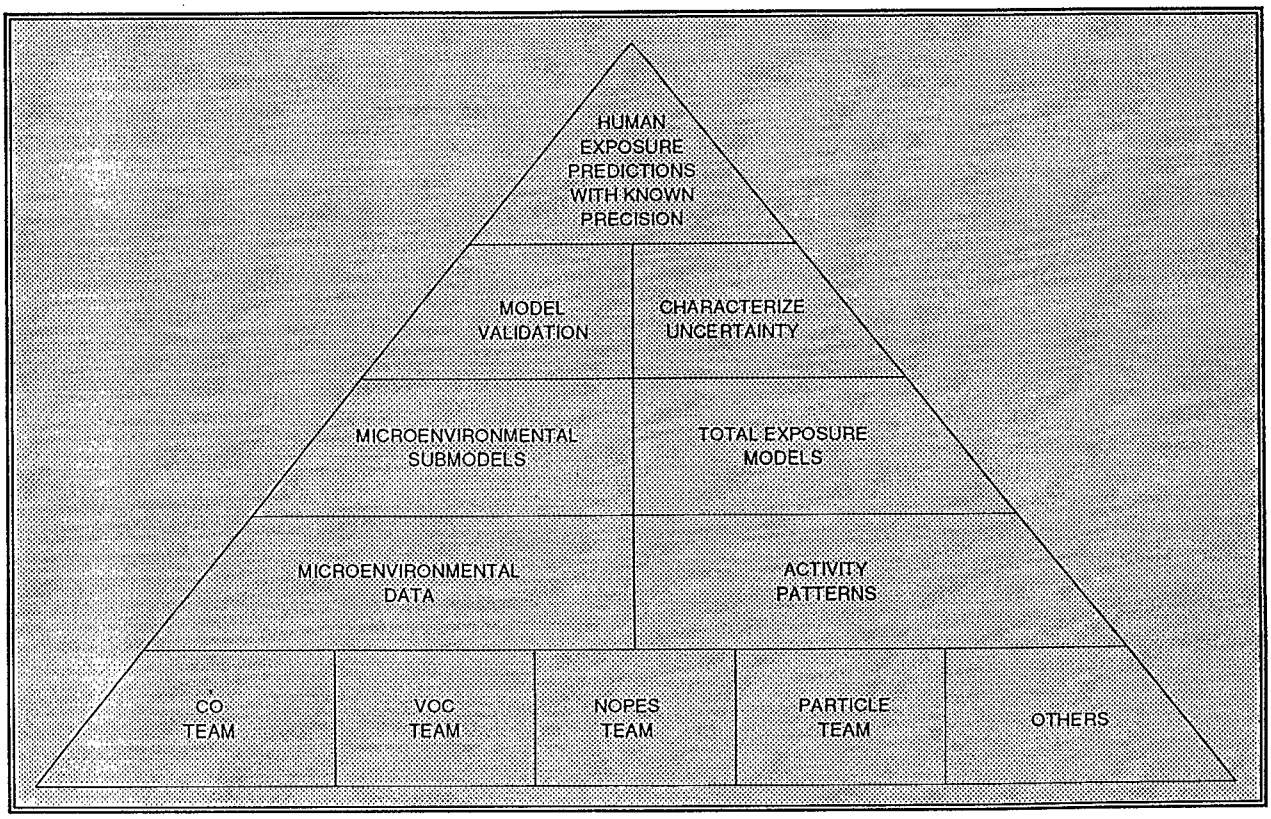

Figure 2-1: Exposure assessment framework.

this two-pronged approach will it be possible to estimate total population exposures to environmental pollutants with a known accuracy and precision. These estimates are essential for the Agency to accurately prioritize the chemicals requiring assessment and to effectively apply a risk-based management strategy in the face of multiple pollutant sources and multiple pathways of exposure.

To generate the exposure and dose estimates necessary for evaluating the routes of exposure by which pollutants reach human beings, appropriate research programs as well as methodologies to assess individual pollutants or classes of pollutants must be developed. The first of these methodologies is virtually complete (for carbon monoxide), and two others are partially developed (for VOCs and pesticides). Many more, however, must be developed—for example, for particles, organics on particles, polar organic compounds, and other regulated pollutants.

At the basis of an integrated exposure assessment methodology is the idea of a three-dimensional bubble around the target human being. If a pollutant in one of the four possible carrier media makes contact with this "bubble," the target is said to have experienced an "exposure" to that pollutant at that instant of time (Figure 2-2). This instantaneous exposure is expressed quantitatively as a concentration (mass/volume) in a particular geophysical medium (mass) at a particular instant of time (time units); and the average exposure is the average of the concentration at the surface of the bubble over some appropriate averaging time. Some pollutants can reach humans through only one carrier medium (e.g., carbon monoxide through air). Others, such as lead and chloroform, can reach humans through two or more media. When multiple pathways of exposure are involved, a person's exposure is the sum of concentrations of the pollutant in each carrier medium at a particular instant of time.

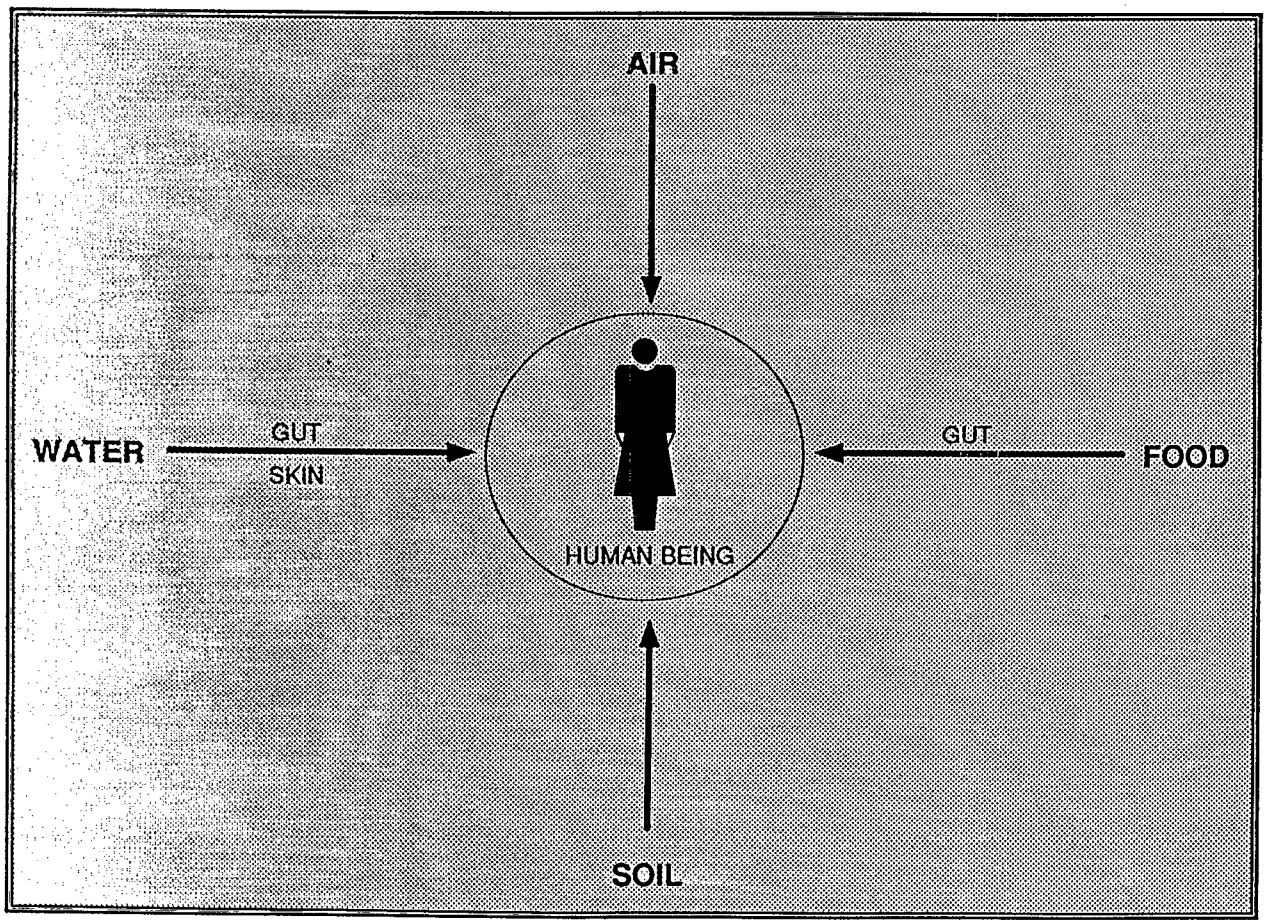

Figure 2-2: Conceptual model of total human exposure.

Once implemented, an integrated exposure methodology for a given pollutant would be used to provide information on the exposures of the target population through all environmental media regardless of the route of exposure (i.e., inhalation, ingestion, or skin absorption). These data will elucidate the number of people exposed, their levels of exposures, and the pollutant sources or other contributors responsible for the exposures. Studies completed over the last few years underline the value of the integrated exposure approach: the findings have already had a great impact on the Agency's policies and priorities in relation to most critical pollutant concerns.

Making the human target the crux of the total exposure model is an innovative approach. Using this methodology, ORD scientists first consider all routes of exposure by which a pollutant may reach a human target, then focus their effort on those particular routes relevant for a particular pollutant of concern. With this structure, they can gather accurate information on the concentrations present and the movement of pollutants through the exposure routes of utmost importance.

Direct and Indirect Measurements Used in Integrated Exposure Assessments

The complementary role of direct and indirect measurements in estimating nationwide human exposures to pollutants is at the basis of any integrated exposure assessment methodology. Direct measurements of the exposure of a target population to a pollutant are taken as follows:

- Selection of a representative and sufficiently large random sample of the population on the basis of a carefully planned statistical design
- Measurement of the pollutant concentrations reaching the population sampled through all relevant environmental media
- Development of estimates, with known precision, about the exposures of the larger population from which the sample was drawn
- Measurement of body burden (breath or blood concentrations) to verify the exposure estimates and infer a dose
- Statistical analysis of information from the respondents' diaries (e.g., activities and locations visited)
- Identification, if possible, of likely sources, microenvironments, and human activities that contribute to exposures

This approach to exposure measurements (i.e., TEAM) is invaluable for determining exposures and sources of exposure for the specific population sampled. EPA has completed a number of successful TEAM field studies addressing carbon monoxide, VOCs, airborne particulate matter, and pesticides. Other such studies are underway.

Indirect measurements, on the other hand, are used to extrapolate data to much larger populations than can be measured directly. Exposure modeling is used to measure and understand the basic relationships between causative variables and resulting exposures, usually in particular microenvironments. These models incorporate data collected in the field, and the exposure predictions derived from them are intended to supplement results from direct studies and to extend and extrapolate these findings to other locales and other situations. Exposure models are not the traditional dispersion models for predicting outdoor concentrations; they are instead designed to predict the exposure of a mobile human being to a particular pollutant. Thus, required model inputs include:

- People's typical activities and time budgets
- Likely pollutant concentrations in places where the people spend time (e.g., home, work, school, in transit)
- Amounts of food and water consumed
- Likely concentrations of the pollutant in question in food and water

An example of a recently developed exposure model is the Simulation of Human Air and Pollution Exposure (SHAPE) model, which was designed to predict exposures of urban populations to carbon monoxide. This model, which is similar to the National Ambient Air Quality Standards Exposure model, is based on the carbon monoxide concentrations measured in a Washington, DC - Denver, Colorado, study that assessed the relative contributions to exposure from commuting, cooking, cigarette smoke, as well as other factors. A model such as SHAPE can be validated by showing that it accurately predicts exposure distributions as directly measured, such as by a TEAM field study. After this validation, the model can be used in a new city—unaccompanied by a field study—to predict that population's exposure to the pollutant in question (the new city's data on

human activities, travel habits, and outdoor concentrations are used as input). In the future, models such as SHAPE are planned for application to pollutants other than carbon monoxide (e.g., VOCs, household pesticides) and for other routes of exposure. The goal of this effort is to estimate total exposure frequency distributions for the entire country, or for major regions.

2.3 PROJECT SELECTION STRATEGY

The general selection strategy for RIHRA projects, described in the introduction to this document, provided the framework from which more specific project criteria in each research area could be developed. For exposure-related activities, the selected projects had to meet these additional criteria:

- A long-term direction and goals consistent with RIHRA objectives
- A cohesive, logical, defensible design
- Likely production of results that would lead to iterative improvements in risk assessment
- Links to the base exposure program (see Introduction)
- Contribution, along with other projects, to a reasonable balance across the RIHRA issues, routes of exposure, and sources of exposure

Table 2-1 provides information on the funded projects.

Under Issue 2.1, researchers will formulate exposure distributions for input into biological human models (see RIHRA Topics 3 and 4). First they will construct submodels for classes of pollutants the Agency regulates by integrating:

- Human activity data
- Existing microenvironmental data
- Source-specific information to account for source/activity combinations with expected high exposures; this information includes exposures to traditional or regulated sources such as incinerators or waste dumps as well as exposures through use of consumer products

Model validation efforts, proposed for FY90-91, will involve the following tasks:

- Comparison of data with exposure distributions derived from total human exposure studies
- Special targeted field studies to identify (fingerprint) sources
- Comparison of data with reconstructive biomarker techniques
- Estimation of the transportability of each submodel for estimating exposures with different exposure or source scenarios

The models will include equations that predict the total exposure resulting from multiple routes. One of the routes that will be examined is the passage of toxic incinerator emissions through the air.

Under Issue 2.2, researchers will compare the activity profiles of populations examined in TEAM studies or other surveys to determine the variation among sampling years and seasons and between geographical regions. This work will be used to assess whether human activity pattern information from one place and time can be used to estimate exposures in a different time and place.

In addition, activities under this issue should show the extent to which existing national activity data can be used for exposure assessment. For example, these activity data should be valid indicators of total time spent indoors but cannot be used to estimate the time spent in activities associated with high exposures (e.g., time spent using specific consumer products). The end product of these projects will be a representative national exposure profile database. That database will then be used as input for the human exposure model; model users could, for example, pick from menus the data from a representative activity/microenvironment/subpopulation that should be included in a particular estimate of total exposure.

Projects under Issue 2.3 address specific information gaps that, according to preliminary information, are sources of variation in exposure estimates. Data on ingestion and short-term peak exposure will be used to refine the submodel components of the exposure model. Researchers will also develop specific detailed exposure distributions for input into pharmacokinetic models, and these data will also be used to interpret the relationship between short- and long-term exposure.

Work under this issue will examine the frequency and impact of pica behavior in children six years old and younger. Other efforts will study the contribution

of household dust and soil ingestion to the exposure of children to metals and pesticides; the data gathered will stand as direct measurements of source contributions that had previously only been modeled. Research in these areas is intended to mitigate an important limitation of past exposure assessments, particularly related to soil cleanup activities, in which large uncertainties regarding household dust and soil ingestion were clearly but unavoidably ignored.

Researchers will also evaluate the contribution to exposure associated with garages in private homes, which have been identified as pollutant sources for organics in indoor air and as possible sources of polycyclic aromatic hydrocarbons. This latter class of chemicals enters homes through the air (doors and cold air intakes) and may also be tracked in on clothing and shoes. The extent to which this source adds to a person's total exposure will be assessed through source characterization of garages and adjoining rooms in the residential environment. Again, data from this work will be used to refine the submodel components dealing with air and dermal/ingestion exposure.

A detailed description of Topic 2 projects is included in Appendix B.

TABLE 2-1

PROJECTS FUNDED UNDER TOPIC 2 EXPOSURE ASSESSMENT

TOPIC/ ISSUE	PROJECT OFFICER	SHORT TITLE	STATUS	FY89 ($K)	DURATION
2.1.1	Irwin/AREAL Behar/EMSL-LV	Model Refinements Benzene Prototype	Coop* - Harvard Univ. UNLV	100	FY 89-91
2.1.2	Schaum/OHEA	Procedures for Evaluating Multimedia Exposures to Incinerator Stack Emissions	Contract: Technical Resources, Inc.	125	FY 89-91
2.2.1	Behar/EMSL-LV	Comparative Analyses of Existing Activity Pattern Databases	Contract: U. MD	150	FY 89-91
2.2.2	Nelson/AREAL	Develop National Database of Activity Patterns for Exposure Assessment	Coop - U. MD	150	FY 89-91
2.3.1	White/OHEA	Pica Ingestion Rates in Children	Coop Fred Hutchinson Research Institute	125	FY 89-91
2.3.2	White/OHEA	Selection of Food Consumption Rates	Pending	0	FY 90-92
2.3.3	Bond/AREAL	Measurements of Exposures to House Dust and Soil Ingestion	Contract: Research Triangle Institute	150	FY 89
2.3.4	Evans	Short-term Spatial Variation of Aerosols and Organics	Contract: Research Triangle Institute	125	FY 89
2.3.5	Highsmith/AREAL	Exposure from Attached Garages	Coop - Rutgers	75	FY 89

*Coop = Cooperative Agreement

Future Directions

Proposed RIHRA exposure activities will be judged by their contribution to a generic model that can predict human exposure profiles through all media using human activity patterns and measured (or modeled) concentrations in significant microenvironments. The iterative process of validating the model, characterizing its performance, and reducing the associated uncertainties will continue until the uncertainties of the model results are not relatively significant to the overall uncertainties of the health indicators. RIHRA offers researchers the opportunity to draw on results of the base exposure programs, formalize these results into an exposure model, and then validate the final product with directly measured personal exposure distributions.

TOPIC 3
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

3.1 ISSUES COVERED

Physiologically based pharmacokinetic (PB-PK) models provide a bridge between exposure data (Topic 2) and biological outcome (Topic 4). In assessing dose and the effects of a given pollutant dose at different levels (e.g., molecules versus tissues) within the body, researchers commonly extrapolate from experimental to real-life human dose levels. The most critical uncertainties associated with this process result from assumptions concerning:

- The comparability of exposure by different routes of administration (route to route)
- The comparability of dose delivery for different regimes of exposure, such as repeated versus single dosing, or episodic, peak, and chronic exposures totaling the same cumulative dose (chronic to acute)
- The proportionality between external exposure level and the resulting delivered dose for higher exposure studies, compared to lower levels typical of environmental exposure (high to low dose)
- Scaling or translation of dose to determine exposures yielding equivalent doses in different species, especially when extrapolating toxic effects in experimental animals to those expected in humans (species-to-species)

PB-PK models are abstract: they represent a system of postulates, data, and inferences presented mathematically to describe the kinetics of the uptake and distribution of chemicals both in laboratory animals and in man. The key physiological variables used in these models are based on underlying biological processes and on the physics governing transport kinetics; thus, using these models can lessen the uncertainty associated with extrapolating from dose to dose or route to route. In addition, species differences are incorporated in the model parameters, so model users can more accurately extrapolate from data gathered from laboratory animals to expected response in humans.

To improve the accuracy of risk assessments, better dose data are needed for input into these models. Such data are necessary to reduce the uncertainties associated with extrapolating from dose to dose, route to route, or species to species. In the past, measurements of **applied dose** (or exposure concentration) have been used instead of data on **delivered dose** (the dose delivered to the tissue or organ of toxic action). The duration, magnitude, and frequency of an exposure, however, greatly affect the delivered dose; and thus the two measurements are not interchangeable.

Examining dose levels, however, does not answer all questions about extrapolation in risk assessment: the equivalency of effects across species is determined not only by relative dose delivery but also by any species differences in reactivity or susceptibility to a given delivered dose. Similarly, the extrapolation of effects from high to low doses or from acute to chronic exposures depends not only on the delivered dose differences in these circumstances, but also on the relative toxicological effects of different degrees and durations of tissue exposure to the proximate toxicant.

Attempts to determine patterns of delivered dose at particular tissue sites focuses attention on the need to understand the mechanisms of action involved. For example, the target site at which the delivered dose should be described may not be clear. Depending on the mechanism of action, the toxic response may be a function of quantity of metabolite formed, the number of adducts or other covalent reaction products formed with crucial cellular macromolecules, the extent of reversible binding to specific receptors, the average and peak toxicant concentration, or duration spent above a crucial concentration. There may be countervailing repair processes of limited or perhaps saturable efficacy. These other factors can and probably do vary among species, strains, sexes, previous histories of exposure, and physiological condition of the subjects.

Thus, although gathering data on dose levels can reduce a great deal of the uncertainty associated with extrapolative procedures, other biological factors must be addressed. Before this step can be taken, however, researchers must account for and eliminate the confounding and obscuring effects caused by dose delivery and pharmacokinetics. Progress in pharmacokinetics is central to the development of biologically based risk assessments.

Issue 3.1: Experimental Absorption and Biological Parameter Data

To improve risk assessments, ORD must gather more experimental and physiological data relative to the effective dose responsible for biological effects. Often both the amount and the biologically active form of the toxicants are unknown. Without data on the absorption, metabolism, transport, and elimination of the parent compound and its metabolites, risk assessors must use uncertainty factors that lessen the accuracy of their assessments. Gathering these experimental data can be coupled with studies on toxic mechanisms of action.

3.1.1 Experimental Absorption Studies

Researchers must balance efforts to develop theoretical models (see below) to predict the uptake and distribution of chemicals with experimental studies in which the uptake, distribution, and time course for elimination of the parent chemical and various metabolites are determined in the major body organs for various species. Moreover, experimental dosimetry studies will support the guidance and design of biological experimentation in a number of organ systems (e.g., reproductive, nervous, pulmonary). Teams should use an integrated approach to encompass oral, dermal, and inhalation exposures for adult, neonatal, and fetal animals, with a linkage to human studies where possible.

3.1.2 Physiological and Anatomical Parameters Across Species

Physiologically based pharmacokinetic modeling provides this key advantage: the model structure is common across species; only the scale is changed. The model developed for one species (along with the attendant insights about delivered dose) can be applied to another species if the various physiological and anatomical parameters governing the kinetics of the compound in the first species are replaced appropriately. Physiological parameters describe capacities and volumes (e.g., organ weights, blood volume, partition coefficients, lung capacity) and rates (e.g., blood flows, metabolic rates, ventilatory rates, elimination rates). Anatomical parameters describe the structure of an organ, such as the size and number of airways in the lung, and the number of glomeruli and the structure of the nephrons in the kidney.

Once most of these values are determined, they can serve as input data in the construction of models for many compounds. Caution must be exercised, however, when making generalizations if any evidence suggests that the chemical being modeled can itself influence the various physiological and anatomical parameters that help to define the model. Metabolic parameters can be very chemical-specific, but even these can benefit by characterization of major biochemical pathways for metabolism of xenobiotic compounds.

3.1.3 Influence of Varying Exposure Parameters (Route, Duration, Rate) on Delivered Dose

Issue 3.1.1 describes experimental dosimetry studies that can be associated with health effects studies. High- to low-dose extrapolation may be highly uncertain due to a lack of pharmacokinetic and pharmacodynamic data. Also, one of the extrapolations that must be made in quantitative risk assessment is from the experimental dose regimen used in an animal toxicological study (repeated dosing or chronic exposure, usually for extended periods) to the expected human exposure patterns (which may be single exposure or chronic, episodic, or continuous). The doses are usually compared on a total cumulative dose basis, e.g., the total mg/kg or ppm/h of exposure. However, both dose rate and dose level can affect the pharmacokinetics of a compound and hence the amount that is delivered to the target site. For example, high dose levels may activate pathways that at lower dose levels do not contribute to metabolic conversions which are linked to the toxicity of the compound.

As work progresses under Issues 3.1.1 and 3.1.2, researchers will begin to examine the influence of route, duration, and rate of exposure on delivered dose in order to understand the uncertainties inherent in extrapolating toxicological data obtained using one exposure scenario and species to another. With data from such projects, teams will develop guidance for improving risk assessment methodologies.

Issue 3.2: Route-to-Route Exposure

Researchers need to identify the assumptions and conditions that affect the scientific defensibility of route-to-route extrapolation. For many chemicals, risk assessments are not available for key routes of exposure because the pharmacokinetic and toxicological data needed to make these assessments are also not developed. What may be available instead is information on the toxicological effects of a chemical associated with a *different* route of exposure. By route-to-route extrapolation, scientists can use the available data to predict exposure by the route of interest. PB-PK models are the best means of getting the maximum use of such data.

Prior to expanding current efforts on route-to-route extrapolation, the Agency will conduct a symposium/workshop to 1) discuss the critical assumptions and limitations related to route-to-route extrapolation; 2) provide specific guidance for risk assessments; and 3) recommend research areas that would facilitate route-to-route extrapolation. Such an effort represents an extension of past activities of organizations like NAS to facilitate the use of pharmacokinetics in risk assessments. Since various EPA Program Offices extrapolate oral toxicity data to inhalation reference doses, the meetings will focus on this issue. Also, dermal versus oral absorption extrapolations are a priority. ORD will seek Agencywide participation in identifying questions for the meetings. Recommendations arising from this exercise will be incorporated into ongoing ORD research programs.

Issue 3.3: Theoretical Models

ORD scientists will explore theoretical models to serve as a unifying structure on which intra- and interspecies dosimetric comparisons can be made. Development of theoretical PB-PK models for chemicals will facilitate better estimates of dose-equivalence across species for various rates and durations of exposure. The development and modeling of pharmacokinetic information from various species will enable researchers to determine the pharmacodynamic differences among species. Improved mathematical formulations will be derived for the disposition of compounds following oral, dermal, or inhalation exposure that incorporate both age- and species-specific input parameters, such as partition coefficients and blood flow, and the properties of the molecules being considered. With validated PB-PK models, researchers can scale exposure, dose, and effects observed in one circumstance (e.g., in laboratory animals in a controlled environment) to completely different circumstances (e.g., human beings in an uncontrolled ambient environment).

3.3.1: Theoretical PB-PK Models

With theoretical PB-PK models, researchers can make intra- and interspecies dosimetric comparisons. Although specific chemicals are often restricted environmentally to only one or two exposure routes, models are needed for all routes of exposure (oral, dermal, and inhalation). A major impetus for the application of pharmacokinetics to risk assessment is the suspicion that experimental rodents may exhibit quite different degrees of delivery of an applied dose to the site of action than humans. This difference would be due to the physiological and metabolic processes in small mammals that occur at much greater rates than in humans. While pharmacokinetic differences are not the factor affecting a chemical's potency in various species, extrapolation procedures should account for differential dose delivery.

3.3.2: Structure-Activity Relationships in Mechanistic Models

The actions of xenobiotic agents in a biological system are a direct consequence of their molecular properties and are produced by a variety of specific molecular interactions and non- specific processes. These agents may interact with receptors, enzymes, and macromolecules involved in transport by fully or partially mimicking the relevant properties of endogenous substances or by evoking the detoxification potential of the biological system. The scientific basis for predicting risk to human health from specific chemicals will be enhanced by pharmacokinetic models that incorporate not only the physiological parameters of the system being modeled, but also the molecular structure and reactivities of the substances involved. The latter information will provide important insight into the transformation, distribution, and deposition of the chemicals under consideration because these properties depend on the biclogical activity of the chemicals. Additionally, because the potential risk from a chemical must often first be assessed without all the relevant data, a modeling approach that incorporates molecular properties has a significant advantage.

3.3.3 Models Linking Exposure to Dose to Biological Outcome

Even after estimates of delivered dose have been made, a great deal of uncertainty still surrounds the incorporation of these estimates into the quantitative risk assessment methodology. The delivered dose information will aid in the extrapolations, but factors outside of pharmacokinetics must also be considered. Species, for example, may have different degrees of response to a given delivered dose—not only because of possible idiosyncratic differences in defenses, but also because the physiological processes affected by the toxic agent are themselves subject to scale differences in different-sized mammals. When focusing on the "biologically effective dose," scientists must also incorporate knowledge of the biological reaction to that dose to make proper use of the pharmacokinetic data. Projects under this issue will examine the interactive role of pharmacokinetic and pharmacodynamic factors. The activities under this issue are linked to those under Issue 4.2.3 (Interaction of Exposure Parameters on Outcome).

3.2 PROJECT SELECTION STRATEGY

The rationale and general strategy that drives research in the area of PB-PK models has been outlined in the Introduction. For PB-PK model-related activities, the selected projects had to meet these additional criteria:

- Long-term direction and goals consistent with RIHRA objectives
- A cohesive, logical, defensible design
- Likely production of results that would lead to iterative improvements in risk assessment
- Cohesiveness of the experimental and theoretical modeling components of the program
- Appropriateness for the desired program mix of projects (i.e., balance of highly relevant and focused short-term projects with immediate delivery of products and of projects that address more generic and long-term RIHRA goals)

The projects can be evaluated on the basis of these criteria as well as the strategy outlined in the Introduction. Table 3-1 provides a listing of projects for FY89 by RIHRA issue.

Under RIHRA Issue 3.1, Experimental Absorption Studies, research efforts provide a balanced approach encompassing oral, dermal, and inhalation routes of exposure. The ORD base program (see Introduction) is surprisingly deficient in experimental pharmacokinetic studies. To meet this recognized need, a Dosimetry Branch was proposed as part of the reorganization of the Health Effects Research Laboratory (HERL). Some of the work under this issue explores research areas that were already being investigated by the base program, and these efforts were redirected under RIHRA support. The ongoing research of the Office of Health and Environmental Assessment (OHEA) into the dermal permeation of compounds bound to soils will also be complemented by RIHRA support. Finally, some research under this issue focuses on obtaining specific PB-PK data for compounds with known multispecies carcinogenic responses.

TABLE 3-1

PROJECTS FUNDED UNDER TOPIC 3
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

TOPIC/ ISSUE	PROJECT OFFICER	SHORT TITLE	STATUS	FY89 ($K)	DURATION
3.1.1	Hoang	Permeation Coefficients	Coop* - UCSF	50	FY 89-91
	Hall	Dermal Absorption	Coop - UCSF Contract NSI	330	FY 89-91
	Gerrity	Particle Deposition/Clearance	Coop - Univ. N. Carolina	130	FY 89-91
	Gerrity	Dosimetry of Aqueous Aerosols	On-Site EMSI	100	FY 89-91
	Gerrity	PK of Inhaled/Ingested VOCs	Coop - Univ. Georgia	117	FY 89-91
	White	PK and Known Multispecies Cancer Potency	To be determined	0	FY 90-92
3.1.2	Farland	Data Base of Biological Parameters	To be determined	15	FY 90
3.2	Gerrity	Route-to-Route	On-Site NSI	75	FY 89-90
3.3.1	Gerrity	Inhalation RFD Methodology	On-Site NSI	0	FY 90-92
	Gerrity	Development PB/PK Models	On-Site NSI	240	FY 89-91
	White	Model Validation and Sensitivity	Coop - Univ. Delaware	50	FY 89-91
3.3.2	Rabinowitz	Computational Models	On-Site EHRT Coop - Molecular Res. Inst.	150	FY 89-91
3.3.3	Kavlock	Embryonic Dosimetry	Coop - NCSU	200	FY 89-91
	Beliles	Biological Time for Teratogenesis	Coop - Childrens Hosp-Cin	70	FY 89-90

*Coop = Cooperative Agreement

Prior to the commitment of large amounts of resources to Issue 3.2, a scientific consensus must be built regarding the priority research concerns. Controversy surrounds a number of questions related to the issue of route-to-route extrapolation:

- How and when is it appropriate to use oral toxicity data to establish an inhalation reference dose?
- What are the assumptions that must be met for dermal to oral extrapolation?
- What minimum data are needed to defend the assumptions that support certain extrapolations?

Thus, RIHRA efforts under this issue are devoted to convening workshops and expert panels to prioritize concerns and to provide research recommendations. Research projects under Issue 3.3 have been designed to complement the experimental studies outlined under Issue 3.1 (i.e., model formulation and validation projects that are designed to build from the experimental data gathered under Issue 3.1). Figure 3-1 depicts the three levels of research associated with developing PB-PK models. Such models are used to:

- Estimate delivered dose
- Perform reconstructive exposure assessments
- Where appropriate, apply the results of biomarker studies to quantitative exposure and risk assessments
- Assist in making route-to-route, dose-to-dose, and scenario-to-scenario extrapolations

Level I contains specific experimental issues (see projects under Issue 3.1) that must be resolved as a basis for pharmacokinetic model formulation and testing. To provide better input for PB-PK models, needs such as the following must be addressed:

- Better and more detailed quantitative descriptions required of the absorption processes at the major portals of entry (e.g., developing good definitions of dermal absorption and how to determine key parameters in dermal absorption)
- Better route-specific descriptions required to increase confidence in extrapolation
- Examination of existing methods for determining model parameters (e.g., partition coefficients, permeation constants, and metabolic rate constants) and guidance for choosing the best method on a case-specific basis
- Determination of the most precise procedures for extrapolating parameter values from one species to another

Level II comprises efforts to formulate a model and apply it to simple exposure scenarios. During this model synthesis phase (see projects under Issue 3.3), the following steps would be taken:

- Establishment of criteria for model testing and validation
- Extrapolation of models for interspecies comparisons (building on Level I efforts)
- Determination of different species parameters by allometric scaling or use of accepted values
- Determination of parameters that do not lend themselves to allometric scaling (e.g., metabolic rate constants) on a case-by-case basis
- Development of criteria and guidance for the interspecies transfer and application of pharmacokinetic models
- Beginning development of higher-resolution models (e.g., for estimating proximate dose near the effective site of action) and of pharmacodynamic models (e.g., for describing not only the concentration of putative toxin but also the alteration of physiologic processes such as neurotransmitter blockage)

At Level III, efforts will be focused on applying the outputs of the first two phases in exposure and risk assessments. In particular, the methods will be tried in more complex exposure scenarios, such as involving mixtures; these tests should highlight additional issues for resolution.

The Agency does not have to prioritize projects at these three levels of model development for completion in a specific order. For example, pharmacokinetic models have already been applied to risk and exposure assessment processes in several situations; and, as a result, several of the issues outlined here for Level I and II research were identified. When these studies are completed, the methods used in these previous assessments will be modified as appropriate. Model research and application is thus an iterative process, not a sequential passage from level to level with a fixed end product.

Significant effort in the base research program has already been devoted to PB-PK modeling for inhaled compounds. Other routes of exposure, however, are under-represented in the program, and only a few HERL scientists have the relevant experience to develop a coherent program in the missing areas. Research, therefore, will provide resources to fill this void through the on-site contractor to HERL. Advantages of this approach include:

- Cost-effectiveness of using the staff depth and capabilities presently available through the contractor (i.e., whole teams of biophysicists, computer programmers, pharmacologists, and toxicologists are needed to develop PB-PK models)
- Importance of the Dosimetry Branch proposed as part of HERL as a focal point for the laboratory on PB-PK research (i.e., because information on dose to target will then be available for observed biological effects, future health effects studies by HERL will become even more useful to risk assessors)

Investigators will also examine structure-activity relationships in mechanistic models. Efforts in this area can help synthesize the assessment of risk through the examination of issues related to the toxicity of chemical classes. The resources allocated to this research area reflect an increased level of effort over base activities, but work under RIHRA will largely deal with classes of compounds that are not presently addressed under the base program. Efforts in PB-PK modeling and structure-activity relationships can also be synergistic: advances in one area can help to focus research issues and objectives in the other. For example, once a structure-activity relationship is established between a tested and untested pollutant, predictions can be made regarding the latter's activity (e.g., partition coefficients) that are based on chemical structure; and once established, these estimates can then be incorporated into a PB-PK model.

To underpin the PB-PK models being developed, researchers will synthesize and develop mathematical and physiological bases for model validation, formula-

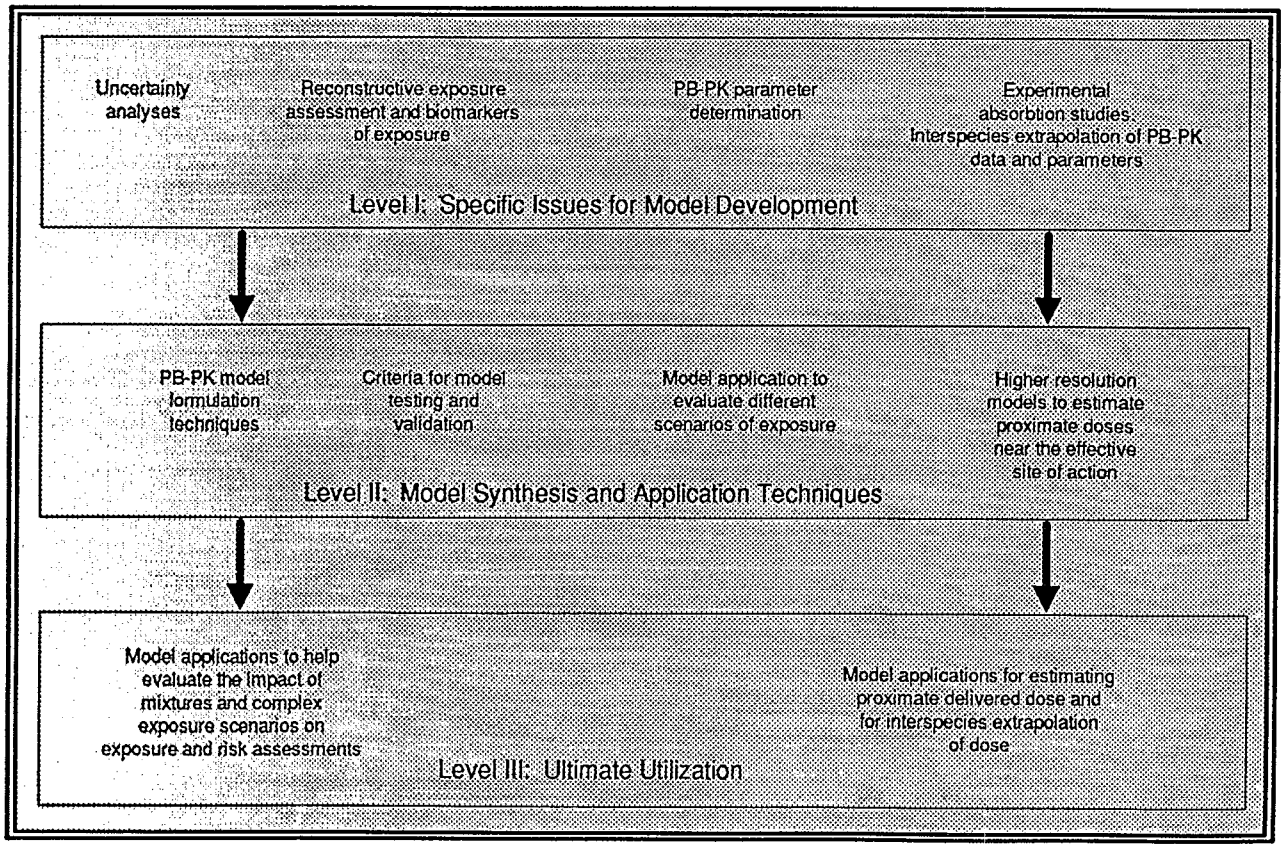

Figure 3-1: Levels of PB-PK modeling research.

tion, and sensitivity analyses. They will also attempt to link exposure to dose to biological outcome—a goal that is key to the RIHRA program. The linkage of exposure-dose-outcome data is necessary as inputs into the risk assessment process; yet, success in this area is difficult to achieve. Interdisciplinary research teams must be brought together and then kept highly focused on a single objective. In the area of developmental biology, some linkage work has been undertaken in the base research program as well as in some RIHRA projects. Working models are being developed to focus interdisciplinary studies on the exposure-dose-outcome linkage.

Because PB-PK models are the bridge between Topics 2 and 4, efforts will be made to link closely the research activities in all three areas. For example, the Topic 2 exposure assessment projects will provide the input driver functions for the PB-PK models that are critical for the models' accuracy and usefulness. The Topic 2 studies, in other words, will apportion the exposure concentration of a given chemical or class of compounds among the various routes (e.g., air, water, soil); and the PB-PK models will then take those input values and apportion the dose of the compound throughout the body. Without an accurate understanding of exposure, there can be no accurate estimation of delivered dose, and thus no accurate understanding of biological outcome. In addition, Topic 2 projects that assess sources of error in exposure models will provide outputs for use as inputs in PB-PK models developed under Topic 3.

Detailed descriptions of Topic 3 projects are provided in Appendix C. Linkages between projects are also highlighted there—for example, Project 3.1.1(1), on dermal absorption of para-substituted phenols, is designed to provide animal and human PB-PK data and is linked to Projects 3.3.1(2), 3.3.2-(1), and 3.3.3(1).

TOPIC 4
BIOLOGICALLY BASED DOSE-RESPONSE MODELS

4.1 ISSUES COVERED

Biologically based dose-response modeling (BB-DR) is the ultimate goal of quantitative risk assessment. By explicitly modeling the underlying mechanisms of toxicity, BB-DR models facilitate these improvements in the risk assessment process:

- **Extension of the observed dose-response relationship to much lower doses.** Low-probability responses cannot be observed in bioassays of reasonable size. Mechanistic models, however, provide a means of relating the quantal dose-response (which cannot be detected when events are rare) to the underlying continuous mechanisms (dose-effect) even down to very low levels (measurement is limited only by the resolution of the analytical techniques).

- **Specific predictions about relative sensitivity across species.** The level of human risk is seldom observable directly; thus, the sufficiency of nonmechanistic cross-species extrapolation methods cannot be established empirically. Mechanistic models, however, allow application of dose-response data on one species to another because they are designed to account for quantitative differences in targets and in the various rates of attack, repair, and propagation of damage.

- **Provision of a framework for generating and testing hypotheses about mechanism.** Models embody current knowledge or hypotheses about underlying mechanisms of toxicity. As such, they provide direction for their own validation and improvement—in other words, the design of the model highlights data gaps that must be filled for further model refinement. For a given set of observations, hypothetical mechanisms can be evaluated by varying the model parameters and then testing the model's ability to predict experimental results. Thus, modeling should proceed in concert with the gathering of data and elucidation of toxic mechanisms.

In recent years, progress has been made in developing mechanistic models as a means of investigating and improving the extrapolations used in risk assessment. Such models describe the key physiological and biological elements and processes, as well as their interactions. Well-constructed models trace the consequences of variation in any key element as it differs among species or dose levels. Mechanistic models provide a means to incorporate new data and theories about special mechanisms of toxic action into quantitative extrapolation. For example, the impact of very different levels of chemical stimulation of peroxisome proliferation in different species could be accounted for by modeling the consequences to the chain of biological processes, i.e., carcinogenesis.

In summary, the advantage of the mechanistic approach is that it allows the overall process of extrapolation to be broken up into its biological elements. Experimental data can be developed as independent elements, and the consequences to the overall extrapolation determined. As knowledge of the underlying processes improves, the biological realism of the model improves, and an experimentally testable basis is developed for extrapolations that must currently be done on the basis of assumptions. The models themselves are generic and are applicable to a variety of chemicals, but they can be adjusted to incorporate chemical-specific effects information (such as metabolic differences across species or particular mechanisms of toxicity).

Under the RIHRA program, this biologic/mechanistic orientation will guide the development of BB-DR models. Research will be directed at determining the conditions under which data obtained in test species and test systems can be used to predict toxicity in humans. Such efforts will reduce the uncertainties associated with existing methodologies as well as lead to the development of new BB-DR models for human health risk assessment. Ancillary components of these activities will be the development of protocols to 1) validate these models, and to 2) facilitate their application in risk assessment.

To develop BB-DR strategies, scientists must both use existing data and generate new data; and the extent to which each option is exercised will, in part, depend on the state of mechanistic knowledge for a given health effect. The issues to be explored in this area—intra- and interspecies extrapolation, extrapolation of health risk across different exposure scenarios, and delineation of multiple, contributory mechanisms of toxicity—are associated with major uncertainties. Research will reduce these uncertainties by confirming or replacing assumptions with scientifically defensible data. Emphasis will be placed especially on pulmonary, reproductive/development, and

neurological health effects, with some effort devoted to cancer effects. Because the primary focus of this research is to better understand the role of various biological processes on chemically induced injury, the models should be flexible enough to incorporate new information as it is obtained.

Issue 4.1: Inter/Intraspecies Extrapolation

Risk assessors find considerable uncertainty associated with the factors that are responsible for differences in response within and across species. Researchers must elucidate the critical physiologic and mechanistic factors that contribute to the health effects of concern in the risk assessment process. Such research will improve the basis on which risk assessors adjust for intra- and interspecies variability in dose-response extrapolations.

4.1.1: Homologous Models

To determine the extent to which effects observed in one species can be extrapolated to another, researchers must ascertain whether effects in animals are analogous (i.e., superficially similar) or homologous (i.e., resulting from a common mechanism of action) to those in humans. Research emphasis will be placed on evaluating species similarities and differences in both mechanism and expression of a given outcome. Thus, work in this area will not only attempt to confirm the existence of homologous mechanisms for inducing specific toxicities (i.e., disease), but also to define the degree of homology in the expression of such disease (i.e., comparable outcome).

4.1.2: Interspecies Sensitivities

Using pharmacokinetic models (Topic 3), scientists will be able to determine the effective dose at a given target site. However, even given equivalent target doses, interspecies differences in sensitivity may still affect the severity of the resulting health effects. Research will focus on the degree to which dose-effect functions for a given health effect (e.g., reproductive failure) differ across species, as well as the degree to which the relative sensitivity of different health effects (e.g., reproductive versus neural) vary across species at a given dose. Efforts under this issue will also determine the appropriate dose metric for expressing and comparing a given dose across species (e.g., mass/unit volume, mass/unit area).

4.1.3: Intraspecies Sensitivity

Work in this area will characterize the factors that may contribute to different sensitivities in response to chemical exposure among individuals of the same species. Variables to be evaluated include age of the individual (developing, adult, or aging organism), previous or current health status, and genetic makeup. Such data will allow for a better estimate of the differential probability and extent of a given health risk for particular subpopulations. An important aspect of this research will be determining how these factors interact with pharmacokinetics to produce intraspecies differences in sensitivity.

Issue 4.2: Exposure Scenarios

Major uncertainties hinder understanding of how variations in dose-rate, concentration, and duration of exposure to environmental pollutants affect toxicological outcomes in humans. Research efforts in this risk assessment issue will determine the effects of varying route, dose, dose-rate, duration, and cumulative dose on health outcomes. Attention will also be directed toward defining the continuum of effects and their toxicological significance as a function of exposure. These efforts will be designed to develop BB-DR models in which both data and assumptions realistically reflect human exposure scenarios. Some activities in this area are expected to be cross-cutting with those under Topic 3 (i.e., characterizing the behavior of the delivered dose under different exposure conditions). Clearly, exposure scenarios are important determinants of outcome for a number of health endpoints. Research in this area is therefore critically important to risk assessment and risk management.

4.2.1: Mechanisms Across Dose

To extrapolate accurately, risk assessors must know whether the mechanism of toxicity varies as a function of dose. Testing protocols that evaluate many toxicological endpoints use some approximation of the "maximum tolerated dose" as their high dose, with lower doses being mathematically reduced multiples of that level. In evaluating dose-response relationships, risk assessors often assume that the mechanism of toxicity does not vary as a function of exposure scenario, and that novel or secondary mechanisms do not influence outcome at these very high exposure rates. Yet, the experimental subjects may be exposed to conditions that might well exceed their capacity to biotransform and excrete the active moiety, might saturate or cause disruption of natural protective/repair mechanisms, and/or might trigger

nonspecific stress responses. Because of the similarity of protocol designs, results from studies on the validity of effects caused by very high dose exposures cut across multiple areas of toxicological significance.

4.2.2: Sensitivity of Endpoints as a Function of Dose

In a dose-response study, endpoints range in severity from biochemical alterations, to physiological changes, to pathological conditions, to mortality. Risk assessors must understand the progression of these biological effects in terms of adaptive responses, compensatory responses, and overtly adverse or pathophysiological responses. Research projects under this issue will define the full extent of responses throughout the experimental dose range, the interrelationships among these effects, and their biological significance.

4.2.3: Influence of Exposure Parameters on Outcome

At one extreme, chemicals exert their effects when a critical body burden is exceeded, regardless of the level and duration of exposure. At the other extreme, the toxicity of a chemical depends on the dose rate or duration of exposure. In the latter case, the toxic effects of a short-term exposure to high concentrations may be very different from those produced by long-term, low-level exposure. In some disciplines of toxicology, this issue translates into a choice between the peak concentration and the cumulative exposure as the trigger for inducing toxicity. Researchers must therefore attempt to improve our understanding of the interplay among rate, intensity, and duration of exposure as they affect toxicological outcomes so that the appropriate trigger can be identified.

Issue 4.3: Mechanistic Variation

A variety of biological events may contribute to the occurrence of a given health effect. Researchers must develop dose-response models that factor in the potential for different biological mechanisms to elicit, initiate, or contribute to the health effects of concern. To date, the primary efforts in this area have focused on delineating the role of non-genetic events in the development of dose-response models for cancer. This effort will continue, and comparable consideration will be given to the variety of mechanistic pathways that contribute to mutagenic events or other target organ toxicities.

4.2 PROJECT SELECTION STRATEGY

The rationale and general strategy that drives research in the area of BB-DR models has been outlined in the Introduction. To explore all of the issues listed there, however, would well exceed available resources for Topic 4 research. Thus, the Office of Health and Environmental Assessment (OHEA) and the Office of Health Research (OHR) have identified and prioritized the research activities that can be addressed realistically with available resources and can provide data that impact on the risk assessment process within a reasonable timeframe.

To facilitate the prioritization process, a decision-tree strategy (see Section 4.3) was developed that could be systematically applied by key investigators in each specialty area (e.g., neurotoxicology or cancer). Groups were asked to consider the critical assumptions for each target system (process) and the importance of resolving those uncertainties based on:

- Impact
  Direct: Research will result in direct modification of current risk assessment practices and/or positions. Work is usually short-term in timetable and application.

  Indirect: Research will provide necessary data for other research efforts that address uncertainties in risk assessment. Work is longer-term in timetable and impact.

- Feasibility (i.e., given limited resources, can the work be done?)

- Timetable for accomplishing the work

The ability to achieve the goals outlined in the Introduction differs across specialty areas as a function of state-of-knowledge. Investigators in all health effects areas, however, were encouraged to:

- Develop a well-articulated basis for the research focus within the area

- Clarify how the proposed work integrates with existing base programs in the respective offices and recommendations on interactions between OHEA and OHR on specific projects.

Since 1986, OHEA has had a prototypic research program on Reducing Uncertainties in Risk Assessment (RURA). Those efforts have been folded into the RIHRA program. Projects funded under RURA are so designated in Appendix D.

4.3 DECISION-TREE STRATEGY FOR PRIORITIZING RESEARCH NEEDS FOR A GIVEN TARGET SYSTEM

A decision-tree strategy (Figure 4-1) was used as one guide in evaluating proposals under Topic 4. The research questions posed represented successive levels of BB-DR model development. Within each discipline, therefore, these levels can be used as a sequencing strategy in choosing the most appropriate projects for immediate funding. For example, a project that is designed to develop a BB-DR model for a target system (Level III) in a test species might not be the best choice for immediate funding if sufficient homology data (Level I) have not been gathered for that test species.

Level I: Assessment of Homology (Qualitative Comparability) of Current Test Species

Work in this area defines the relative merits of the current test species in terms of:

- Comparability of basic physiological processes to humans
- Homology of endpoints to humans

This qualitative concern must be addressed before the test species can be used in more quantitative research. This research area should not be confused with work in species-species extrapolation, which is directed at calibrating species differences between an acceptable animal model and humans.

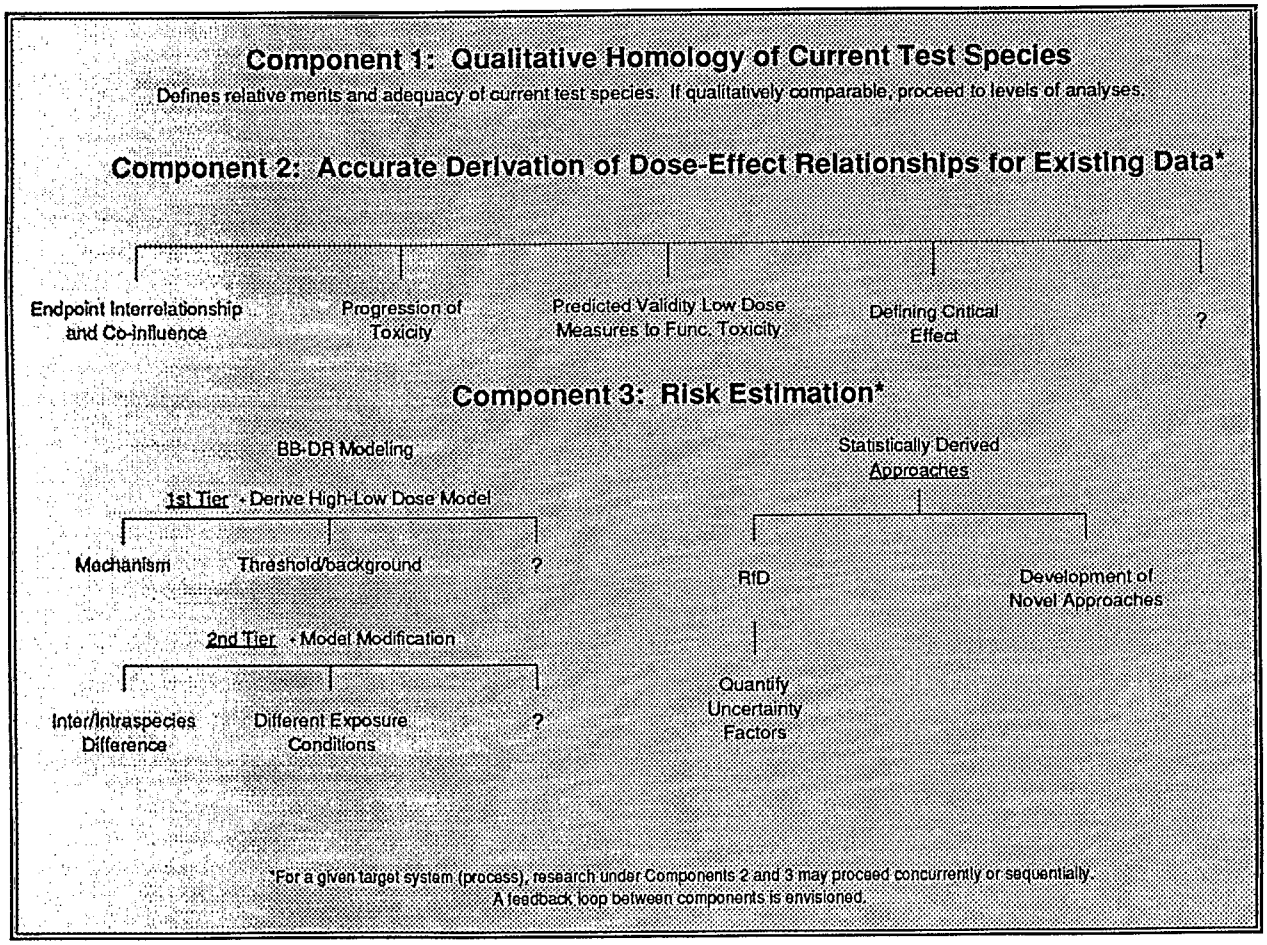

Figure 4-1: Components of strategy to be applied to a given target system (process): developing research rationale and focus.

Level II: Accurate Derivation (Expression) of Dose-Effect Relationships for Existing Data

Work in this area focuses on developing an empirical base of data on underlying mechanisms and their toxicological consequences for a given target. Uncertainties in this area concern identifying, analyzing, and expressing existing data in a manner suited to deriving the most accurate and relevant dose-effect function for the toxic event. Examples of possible activities within this area include:

- Endpoint interrelationships and co-influences on the expression of the dose-response curve
- Identification of mechanisms/processes that affect the progression of toxicity across dose
- Predictive validity of low-dose sensitive measures to higher-dose functional outcomes
- Integration of the previous three types of activities to define critical effect

The primary goal of work in this area is the establishment of empirical relations between component processes. Data must be gathered, therefore, not only on dose-effect relationships but on **effect-effect relationships** (i.e., the influence of underlying processes on one another) and on **effect-response relationships** (which empirically tie different levels of the underlying continuous physiological effects to the probability of quantal response). Results from projects in this area would feed back on the qualitative models (by validating or refuting causal links) and would also be the basis for generalization, parameter estimation, and testing of the quantitative models.

Research in this area may have the greatest impact (see the criteria listed at the beginning of this section) of any work under Topic 4. Possible direct impacts include better guidance to Program Offices concerning the appropriateness of a given endpoint on which to base a risk assessment, as well as better control of the RfD process by clarification of the term "critical effect." Possible indirect impacts include provision of data for guiding the development of BB-DR models.

Level III: Quantitative Approaches to Risk Estimation

Efforts within this area would be concentrated on reducing the uncertainties associated with the application of either statistical approaches (e.g., RfD) or BB-DR models for risk estimation by use of the empirical relationships identified above. These activities are interdependent.

Statistical Approaches

These approaches are likely to continue to dominate the Agency's risk assessment process for noncancer health effects. Therefore, research that reduces the uncertainties associated with these methods would have a direct impact on the process of risk assessment. Projects of this sort usually have relatively short timetables. Work following this approach falls into these categories:

- RfD process: studies to calibrate inter- and intraspecies differences to provide statistical certainty concerning the appropriateness and magnitude of the uncertainty factors
- Development/application of novel mathematical approaches: studies do not rely only on the No Observed Adverse Effect Level (NOAEL); instead, they use total dose-response data in linear extrapolation and attempt to define the dose associated with specific risk levels (e.g., benchmark approach)

BB-DR Models

Work in this area requires longer-term support than statistical efforts. Although modeling projects can continue over long period of time, their intermediate stages provide much potential benefit to the risk assessment process. Following is a possible tiered approach to model creation:

1. Derive a "working" high-to-low-dose extrapolation model(s) for a given target process; required efforts include:
   - Identification and integration of existing basic biologic information
   - Support of work on mechanisms to identify critical biologic processes
   - Verification/integration of threshold for an outcome into the BB-DR model
   - Other efforts not yet defined
2. Use the model to define (and drive) subsequent research designed to address data gaps and recalibrate the model

3. Adjust the model for inter- and intra-species differences and different exposure conditions

It should be noted that some of the data required under tier 3 (e.g., interspecies differences) may be available from studies conducted under RIHRA Topic 3 (Pharmacokinetics).

4.4 SUMMARY OF THE PROPOSED RESEARCH PROGRAM

Under Topic 4, research programs have been funded in the following areas:

- Neurotoxicology
- Developmental/reproductive toxicology
- Pulmonary toxicology
- Genetic toxicology/cancer
- Generic, cross-cutting issues dealing with epidemiology, severity of effects, duration of exposure, and improving the RfD process

Groups responsible for each area were asked to prepare a statement of focus and rationale as the basis for prioritizing their projects. The rationales for project choice in the areas of genetic toxicology/cancer and reproductive/developmental toxicology were developed jointly by OHR and OHEA. The Topic 4 co-chairs, Drs. Robert Kavlock and Harold Zenick, then used the program packages (including overview, prioritization chart, and project descriptions of existing and proposed work) to recommend the most important projects for Topic 4 resources.

During this final prioritization process, the following considerations were recognized:

- Several of the proposed projects would provide important data for a number of Topic 4 issues; thus, assignment of the projects to a given issue was occasionally arbitrary (see Table 4-1).
- The rationale statements for programs on the various target systems had to include projects already incorporated into the RIHRA base program (or the OHEA RURA program) as well as new proposals. The emphasis and priorities of the programs were therefore often shaped by these prior commitments. Presumably, as the RIHRA program evolves, these constraints will lessen.
- The nature of the projects proposed under Topic 4 suggested a number of possible interactions between OHEA and Health Effects Research Laboratory (HERL) personnel. These interactions are being pursued.

Following is a brief synopsis of the programs for each research area. The detailed rationale and project descriptions for each of the areas noted here may be found in Appendix D. See Table 4-1 for a listing of the funded projects under Topic 4.

Neurotoxicology

The majority of RIHRA projects in this area concentrate on inter- and intra-species comparability, especially in terms of sensory and cognitive processes. Related work compares various quantitative approaches to the RfD in estimating neurotoxic risks. Additional projects address the influence of exposure conditions on outcomes.

Several of the proposed projects—especially the work examining quantitative models and evaluating historical pharmaceutical databases—can provide excellent vehicles for interaction between OHEA scientists at ECAO-Cin and HERL. ECAO-Cin has substantial experience in the areas of modeling and the use of existing databases; NTD-HERL has the expertise to evaluate the scientific credibility of various approaches to neurotoxicity data.

Developmental/Reproductive Toxicology

HERL and OHEA groups have joined in developing and evaluating reproductive and developmental risk assessment guidelines for focusing research activities in this area. Under female reproductive risk assessment, research efforts are addressing issues concerning the homologous mechanisms associated with processes of ovulation through implantation. Under male reproductive risk assessment, efforts are concentrating on defining endpoint relationships and determining the functional significance of low-dose measures of toxicity.

The developmental segment of the program addresses endpoint issues, especially as related to maternal and developmental toxicity. Because a more substantial database is available on developmental concerns, efforts are also being directed toward the development of BB-DR models that incorporate threshold concepts.

TABLE 4-1

PROJECTS FUNDED UNDER TOPIC 4
BIOLOGICALLY BASED DOSE-RESPONSE MODELS

TOPIC/ ISSUE	PROJECT OFFICER	SHORT TITLE	STATUS	FY89 ($K)	DURATION
Neurotoxicology					
4.1.1	Hudnell[b]	Homologous Sensory Function	Northrop Services, Inc.	122	FY 89-90 (91)[a]
	Stanton[b]	Homologous Learning	Northrop Services, Inc.	85	FY 89-90 (91)
	Stanton[b]	Homologous Learning	Coop* - UNC	110	FY 89-90
	Padilla[b]	Peripheral Neuropathy	Virginia Polytechnical Institute	225	FY 89-90 (91)
4.1.3	MacPhail[b]	Suceptibility to Neurotoxicants	Northrop Services, Inc.	81	FY 89-90 (91)
	MacPhail[b]	Quantitative Assessment	IAG† - Natl. Inst. Mental Health	120	FY 89-90
	Jensen[b]	Cholinergic Aging	Coop - UNC Coop - N.E. Louisiana State Univ.	160	FY 89-90
4.2.3	Crofton[b]	Exposure Scenarios	Northrop Services, Inc.	106	FY 89-90 (91)
Developmental/Reproductive Toxicology					
4.1.1	Cummings[b]	Early Pregnancy Loss	Northrop Services, Inc.	106	FY 89-90 (91)
	Gray[b]	Probability of Infertility		56	FY 89-90 (91)
	Cooper[b]	Control of Ovulation	Northrop Services, Inc.	131	FY 89-90 (91)
	Kavlock[b]	Renal Mechanisms	Coop - Duke	125	FY 89-90 (91)
4.1.2	Rogers[b]	Embryo Culture	Northrop Services, Inc.	152	FY 89-90 (91)
	Laskey[b]	Leydig Function	Coop - Johns Hopkins	100	FY 89-90 (91)
4.1.3	Rogers[b]	Litter Effects	Exist NSI/NRC	106	FY 89-90 (91)
	Chernoff[b]	Maternal Genome	Natl. Research Council	66	FY 89-90 (91)
4.2.1	Miller[b]	Maternal Toxicity	Northrop Services, Inc.	71	FY 89-90 (91)
	Chernoff[b]	Low Dose Thresholds	Natl. Research Council	50	FY 89-90 (91)
	Rogers[b]	Zinc Metabolism	Coop - UC Davis	75	FY 89-90 (91)

* Coop = Cooperative Agreement
† IAG = Inter-Agency Agreement

TABLE 4-1 (Cont'd.)

TOPIC/ ISSUE	PROJECT OFFICER	SHORT TITLE	STATUS	FY89 ($K)	DURATION
	Kimmel/ Kavlock[d,e]	BB-DR Models	IAG - Natl. Center Toxicol. Res.	80	FY89-90 (91)
	Dellarco[e]	Zygote Toxicity	IAG - Oakridge Natl. Lab	80	FY 89-90
4.2.2	Kimmeld	Multiple Endpoints	IAG - Hlth. Tox. Prog.	40	FY 89-90 (91)
	Clegg[e]	Sperm-Fertility Relationship	Coop - Georgetown	100	FY 89-90 (91)
Pulmonary[f]					
4.1.2	Hatch	Ozone Dosimetry	Coop - UC Davis	75	FY 89-90 (91)
4.1.3	Hatch	Lung Injury	Exist NSI	265	FY 89-90 (91)
4.2	Koren	Inflammatory Response	Exist Environmental Monitoring Services, Inc.	171	FY 89-90 (91)
Genetic Toxicology					
4.1.2	Nesnow[b]	Rodent/Human Sensitivity	Exist Environmental Health Research Testing, Inc.	136	FY 89-90 (91)
	Moore[b]	Mutation Induction	Coop - U. Vt.	75	FY 89-90 (91)
	Nesnow[b]	AZQ Adducts	Coop - Baylor	290	FY 89-90 (91)
	Farland[e]	Fish Cancer D-R Models	EPA-Duluth	60	FY 89-90 (91)
	Mass[b]	Oncogene Activation	Coop - Med. Coll. Ohio	0	Starts FY 90-91
	Daniel[b]	Hepato/Nephro Carcinogens	Coop - Northern Kentucky Univ.	0	Starts FY 90
4.1.3	Bayliss[d]	Cohort Survivorship	IAG - NIOSH	40	FY 89-90
4.2.2	Dellarco[d]	DNA Alkylations	Coop - Coriell Inst.	95	FY 89
4.3	Mass[b]	Moolgav./Knud.	Coop - Univ. Wisconsin-Madison	170	FY 89-90 (91)
	Nesnow[b]	Gene Expression Alterations	Coop - U. Pgh.	120	FY 89-90 (91)
	Chen[e]	Chromosomal Translocation	Proposed Coop	80	FY 89-90
	Reese[e]	Initiation/Promotion	IAG - FDA	30	FY 89-90 (91)
	Chen[d]	Class of BB-DR Models	Varied	105	FY 89-90 (91)
	Bayard[d]	D-R Promotors/Initiators	Varied	105	FY 89-90 (91)
	Schoeny[d]	Carcinogen Combination	On-Site Cntrt	40	FY 89-90 (91)

TABLE 4-1 (Cont'd.)

TOPIC/ ISSUE	PROJECT OFFICER	SHORT TITLE	STATUS	FY 89 ($K)	DURATION
Cross Issue/Cross Target					
4.2.2	Swartout[d]	Quantifying RfD Process	Varied	110	FY 89-90 (91)
	DeRosa[d]	Less-Than-Lifetime Exposure	Varied	128	FY 89-90 (91)
	Everson[b]	Human Data	SRA	80	FY 89-90 (91)
	Hertzberg/Davis[e]	Severity of Effects	Varied	140	FY 89-90 (91)
	Farland[d]	Workshops Risk Assessment	Varied	150	FY 89-90 (91)

[a]Year in parenthesis denotes major review of progress prior to further commitments of funds in that year.
[b]OHR lead
[c]OHEA lead under RIHRA
[d]OHEA RURA component
[e]Co-funded by OHEA and OHR
[f]OHR lead on all pulmonary projects

Pulmonary Toxicology

Efforts in this area focus on improving the dosimetric and concentration-time relationships in animal and human exposure models. The proposed studies add critical components to existing studies by enabling determination of target tissue dose in nonhuman primates exposed to ozone. Combining these data with those already obtained in rat studies will facilitate extrapolation of a body of existing toxicological data to humans. Other studies are evaluating a recently proposed model of concentration time-by-time relationships for air pollutants through analysis of bronchoalveolar lavage proteins as well as pulmonary host defense systems. These sensitive indicators of damage and repair capability provide a basis from which to develop defensible regulations for a variety of exposure scenarios.

Genetic Toxicology/Cancer

The primary issues in this area are:

- Mechanisms of action
- Interspecies sensitivity
- Biological modeling

Most of the new work focuses on somatic cells. Whereas the FY88 HERL-RIHRA projects concentrate heavily on interspecies extrapolation, the new work is directed toward the definition of basic mechanisms (genetic and nongenetic) of cancer. Special emphasis is placed on the identification of the events of initiation and promotion.

This program represents ongoing discussions between HERL and OHEA scientists and offers great potential for the types of interaction envisioned under the RIHRA program, namely 1) a feedback loop between modeling efforts to identify data gaps; and 2) laboratory work aimed at elucidating basic physiological processes.

At the present time, no projects on endpoints used in heritable mutagenesis risk assessment are included in the program. The critical needs in this area must be better delineated and coordinated with ongoing, substantial programs such as the National Toxicology Program (NTP). A heavy commitment of resources is required to address these issues; a more profitable approach may be to interact with and contribute to the research activities of the NTP program. Research identified in heritable mutagenic

risk will be incorporated into the reproductive and developmental research strategy.

Generic, Cross-Cutting Issues

Activities in this research area address a number of issues with relevance across target systems (processes), including:

- Comparing clinical, epidemiological, and experimental databases to improve species extrapolation
- Convening workshops to address specific issues in the development and implementation of the RIHRA program
- Developing improved methods for quantifying risk (including the RfD)
- Factoring severity of effect and varying exposure conditions into the risk characterization process

These issues are pivotal to any health risk assessment. The research effort will benefit from greater involvement of HERL/OHEA scientists. Special consideration will be placed on target systems for which the expertise is available within HERL/OHEA to closely scrutinize model assumptions, approaches, and results.

The OHEA/RURA program will fund the Workshop activities listed on Table 4-1. This project has been listed under Topic 4; however, these funds are presumably available for workshops addressing any of the four Topic areas.

APPENDIX A
TOPIC 1: ANALYSES OF UNCERTAINTY IN RISK ASSESSMENT PROJECT DESCRIPTIONS

Topic: Analyses of Uncertainty in Risk Assessment

Issue: Research Strategies

Status: New RIHRA project

Title: Review of "Guidelines for Quantifying Uncertainty in Risk Assessment"

Description: General guidelines for quantifying uncertainties in risk assessment are regarded as a high priority issue among Agency risk assessors. Resources for the Future (RFF) has developed such guidelines using input from Federal and academic scientists. In addition, they are applying these guidelines to case studies of current interest to the Agency. This project will use the cooperative agreement with the newly formed Committee on Risk Assessment Methods (CRAM) of the National Academy of Sciences to perform a detailed peer review of these guidelines. A select committee of knowledgeable scientists will be convened by CRAM with input from a Federal Liaison Group. A written report of the committee's findings will be provided to the Agency.

Identified Results: A report of the comments of NAS peer reviewers will be presented to the Agency at the completion of the review. This report will take the form of a project summary but may be developed into a journal article depending on the nature of the review and comments developed.

Usefulness of Results: The Agency will benefit from the efforts of RFF to develop guidelines for uncertainty analysis. In addition to providing input in the development of these guidelines, this project will allow the guidelines to receive necessary scientific review and consensus, which will be needed should the Agency choose to incorporate these guidelines by reference into Agency documents. This project also illustrates the concept of leveraged funding that will be important in carrying out research in uncertainty analysis under Topic 1 of RIHRA.

Project Length and Cost: 1 yr FY89: $35K

Project Officer: W. Farland (OHEA) (202) 382-7315

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Topic: Analyses of Uncertainty in Risk Assessment

Issue: Research Strategies

Status: New RIHRA project

Title: Addressing Uncertainties for an Integrated Exposure Model

Description: The goal of this project is to develop a strategy for evaluating the sources of and characterizing the uncertainty in a TEAM-type of exposure scenario. The project will use tetrachloroethylene in the air and ground water in a hypothetical community as an illustration of the approaches to be applied. This project will be closely coupled with the project described below (Issue: 1.1.2) in its dealing with the question of uncertainties in projecting individual versus population exposure levels.

Transport Modeling. Using multimedia transport models such as GEOTOX, and single media models for air and ground water dispersion in combination with measured data, sources of the chemical will be quantified and relative magnitudes of uncertainties associated with both the multimedia and single-media estimates of contaminant concentrations attributable to a regional source for steady-state and dynamic conditions will be characterized. This approach will allow the apportionment of estimated uncertainty among contributions of model assumptions, transport properties, and chemical properties.

Personal Exposure. For the compound under study, methods for characterizing pathway exposure factors (PEFs) will be identified and uncertainties surrounding each PEF will be characterized. Special attention will be paid to characterizing those PEFs that are both highly uncertain and apparently important contributors to total exposure. TEAM data developed by the Agency will be compared with an integrated exposure model approach developed by LLNL. Uncertainty contributions from physical and chemical properties, variations across microenvironments (such as within households), and variations among individuals are expected to be the focus of this effort.

Identified Results: Results of such a study, combined with Agency research efforts in exposure assessment under the ORD base and within RIHRA Topic 2, will provide a case study of application of the principles of uncertainty assessment currently in use. These data will be presented in a report to the Agency for use in evaluating the application of principles of uncertainty assessment. Depending on the results of the study, and comments during review, the report may be published in the peer-reviewed literature.

Usefulness of Results: Results of this effort will integrate nicely with ongoing EPA work. The case study chosen will have direct applicability to ORD's support for the Air Program in understanding and characterizing exposures for volatile organic compounds, a high-priority issue. This project will leverage work accomplished by ORD and will permit comparison between collected data and model predictions.

Project Length and Cost: 1 yr FY89: $90K

Project Officer: W. Farland (OHEA) (202) 382-7315

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Topic: Analyses of Uncertainty in Risk Assessment

Issue: Research Strategies

Status: New RIHRA project

Title: Interindividual Variability and Uncertainty in Risk Assessment

Description: This project will focus on the element of interindividual variability in the overall uncertainty framework and will characterize the implication of this parameter on uncertainties in the case study under Project 1.1.2. This study will focus particularly on the issue of added uncertainty in estimating individual risks as opposed to population risks, given a range in interindividual variability.

Major Sources of Uncertainty. This project will address the major sources of uncertainty within the post-exposure portions of the tetrachloroethylene assessment. Residual uncertainly and interindividual variability will be assessed quantitatively and characterized using a model developed at LLNL. This final characterization will specifically address the predicted uncertainty and interindividual variability distributions of risks to exposed individuals and the distribution of uncertainty in corresponding population risk (i.e., in the total number of predicted cancer cases).

Communication of Uncertainties. Results of the previously described work will be framed into a readily understandable format, highlighting the distinction between interindividual variability and other risk assessment uncertainties, on the one hand, and between individual risk and population risk on the other. Novel approaches, both analytical and graphical, will be employed for this effort.

Identified Results: Results of this project will be useful to the Agency in addressing two issues within the framework of uncertainty in risk assessment: interindividual variability and linguistic imprecision. The interaction of the two investigators from LLNL will provide a comprehensive evaluation of uncertainty in the case study. As described above, this case is important for the Air Program.

Project Length and Cost: 1 yr FY89: $90K

Project Officer: W. Farland (OHEA) (202) 382-7315

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Topic: Analyses of Uncertainty in Risk Assessment

Issue: Research Strategies

Status: New RIHRA project

Title: Evaluation of Communication of Uncertainty in Risk Assessment

Description: This project will provide sound survey information on how carcinogen classification systems are interpreted by both the lay public and the scientific community. Both presently used descriptors of cancer hazard and those under consideration for future use will be analyzed. In addition, a selection of approximately a dozen case chemicals will be used to explore the practical application of these descriptors to weight-of-the-evidence situations. Groups of scientists will be selected and briefed using approaches validated in earlier work at Harvard involving the influence of expert scientific judgment in cancer risk assessment. This project will be funded co-operatively with Dow Chemical company.

Identified Results: This project will result in a report that will provide insights concerning linguistic imprecision in currently used terms for describing cancer hazards. Depending on the results of the research and review comments, the report may be submitted for peer-reviewed publication.

Usefulness of Results: These results will be useful to the Agency as it re-considers approaches to classifying and describing cancer hazards. Many consider this aspect of the risk assessment process to be the most illustrative case of linguistic imprecision. Survey data will assist the Agency in deciding how it might improve communication of cancer hazards under varying weights-of-the-evidence. The project represents a joint effort between the Agency and industry to address this issue.

Project Length and Cost: 1 yr FY89: $35K (plus $20K provided by Dow)

Project Officer: W. Farland (OHEA) (202) 382-7315

APPENDIX B
TOPIC 2: INTEGRATED EXPOSURE ASSESSMENT PROJECT DESCRIPTIONS

Topic: Integrated Exposure Assessment

Issue: 2.1 Human Exposure Models

Status: New

Title: Modeling Human Exposure

Description: The goal of this project is to improve the accuracy of predictions of exposure for chemicals of interest to the Agency. Human exposure models employ computer algorithms to combine the concentrations of pollutants people experience in various microenvironments (microenvironmental exposures) with the times spent in those microenvironments (human activity patterns). Although human exposure modeling is in its infancy, several prototype models (e.g., SHAPE and NEM) have been developed based on the early concepts developed by Fugas and Duan. To be effective, such models must include all the microenvironments that people normally visit (for example, homes, stores, schools, offices, work places, subways, buses, and automobiles) and must include multiple routes of exposure (air, food, skin, and drinking water). Although several models have been developed, none has been fully validated with real exposure data collected from human exposure field studies. Such validation is necessary to determine the accuracy of the models and to calibrate and redesign them to make predictions with known confidence intervals. Recent findings from total exposure studies suggest how these models can be developed further and improved.

Research on this topic will proceed by first constructing submodels for important pollutants that EPA regulates (selecting, for example, from respirable particulates, volatile organic compounds, semivolatile organics, formaldehyde) using the best microenvironmental exposure field data available. Existing data bases and research literature will be reviewed to construct these microenvironmental submodels, and ongoing microenvironmental field study data that can be used will be carefully evaluated. Serial autocorrelation problems in microenvironmental submodels will be addressed (RTI/Harvard and NOAA).

Simultaneously, the best human activity patterns data currently available (for example, John Robinson's 1986 national activity pattern survey, the 1988 California activity pattern survey, and the activity surveys conducted in Cincinnati, Denver, and Washington, DC) will be evaluated and coded for input into the model (UNLV).

The human activity pattern data and resulting microenvironmental submodels will be combined in a human exposure model using a generalized exposure equation. Once developed, this model will be validated by testing it with field data collected in total exposure field studies for a variety of situations. Uncertainty ranges around the predictions will be characterized (UNLV).

Identified Results: **Short term:** Evaluation of existing models and needed improvements.

Long term: A generalized human exposure model and submodels that have been validated and the uncertainty well characterized. The written product will consist of a

research report and several peer-reviewed articles describing the model, its validation, and applicability to various exposure situations.

These results can be used by all Program Offices in the agency that are involved in estimating human exposures.

FY90 - reported results on modeling the impact of outdoor ambient air concentrations on measurements of total human exposure.

FY91 - reported results on the status of existing human exposure models.

FY92 - reported results on the prototype human exposure model combining multiple pathway exposures.

Usefulness of Results: An essential component of the risk assessment process is the estimation of human exposure to chemicals. This model will improve Agency risk assessments by providing a tool that will permit more accurate exposure assessments than previously possible. This model also will allow the effect on risks to health of different Agency regulatory strategies to be evaluated more accurately.

Project Length and Cost: 3 yr FY89: $100K

Project Officer: Irwin (OMMSQA; AREAL-RTP) (919) 541-4567
Beher (OMMSQA; EMSL-LV) (702) 798-2216

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Topic: Integrated Exposure Assessment

Issue: 2.1 Human Exposure Models

Status: Ongoing

Title: Procedure for Evaluating Multimedia Exposures to Incinerator Stack Emissions

Description: The goal of this project is to improve the process of modeling exposures by developing and applying a standard PC computerized approach. Each year both commercial and social activities in the United States produce more than 150 million tons of discarded waste. Both hazardous and municipal solid wastes have been traditionally disposed of in land areas dedicated to that purpose. Meanwhile, as the amount of wastes needing disposal continues to grow with the increase in the U.S. population, restrictions concerning land disposal are becoming more evident. As ocean disposal and land disposal of wastes are being phased out, waste incineration becomes a more viable solution. Incineration of wastes, both municipal and hazardous, is in most cases not 100% efficient in destroying contaminants. Consequently, deposited stack emissions are drawing increasing attention because deposited contaminants can cause potential health hazards and can create multimedia pathways for human exposure. Deposited contaminants can contaminate soil and the food chain. Runoff from contaminated soil can cause contamination of sediment and water. Example facilities that can be of concern include hazardous waste incinerators, sludge incinerators, lead smelter emissions, and municipal waste incinerators.

A significant effort in the area of multiple pathway exposure analysis due to municipal waste combustor emissions has been developed by EPA's Office of Air Quality Planning and Standards and the Environmental Criteria and Assessment Office in Cincinnati, OH. A draft document entitled "Methodology for the Assessment of Health Risks Associated with Multiple Pathway Exposure to Municipal Waste Combustor Emissions" was released in October 1986. The methodology consists of a series of environmental fate and transport models that utilize the known physical and chemical properties of specific pollutants to predict the:

- atmospheric dispersion from stack emissions
- potential for surface deposition and accumulation
- movement of the settled pollutants through and into various environmental media
- potential bioaccumulation of pollutants into trophic systems
- potential for adverse effects on the vitality of natural ecosystems
- potential for adverse effects on human health

With regard to evaluation of human health effects, the methodology estimates health risks that result from inhalation of predicted ambient air concentrations of pollutants, ingestion of pollutants deposited on the ground and bioaccumulated through the food chain, ingestion of potable water or aquatic organisms contaminated by surface runoff and leaching and percolation of settled pollutants into water supplies, and ingestion of soil contaminated by deposited incinerator emissions.

The final document will address these items: 1) a review of the present methodology, closely coordinating with ECAO-Cincinnati, to ensure that the latest analytical tools are being used in the development of this project; and 2) a user-friendly PC program that will incorporate much of the methodology of the ECAO document which has not yet been programmed in a easy-to-use fashion.

Identified Results:

Short term: A review of the present methodology.

Long term: A computer model that quantifies exposure assessment procedures applied to several point source categories.

FY90 - reported results of the review of the current methodology.

FY91 - PC program for use in evaluating risks from incinerator stack emissions.

FY92 - PC program for use in evaluating risks from other emission sources.

Usefulness of Results: The final product should achieve the following results:

- create a standard procedure to evaluate human exposure to deposited toxic contaminants originating from stack emissions
- create a simplified PC model that will generate exposure and risk values under numerous exposure scenarios for many different levels of stack emissions.

Project Length and Cost: 3 yr FY89: $125K

Project Officer: J. Schaum (OHEA-EAG) (202) 382-8909

Topic: Integrated Exposure Assessment

Issue: 2.2 Human Activity Patterns

Status: Ongoing

Title: Comparative Analysis of Existing Activity Pattern Data Bases and Relationship to Specific Microenvironments

Description: The goal of this project is to utilize existing activity pattern data for predicting human exposure. Human activity patterns are important for making exposure assessments, but EPA has not adequately utilized past activity pattern data bases. Additional work is required to review, interpret, code, assess, and understand activity pattern data bases collected in previous field investigations in the U.S. and Europe.

This project will obtain and examine the data bases collected in activity pattern surveys conducted in the previous five years to characterize differences in urban, state, and regional activity patterns through comparisons with the activity patterns in cities where TEAM studies have been carried out. The data bases selected for review include the Robinson 1986 nationwide activity pattern survey, various urban and statewide activity pattern studies, and European time budget studies.

Improved exposure predictions can be made if the following questions can be answered:

1. In what activity is an individual involved?
2. Where and when is the activity taking place in time and space?
3. What is the duration of the activity?

This research will associate each activity with a frequency distribution of microenvironments in which the activity occurs. A detailed stratified structure of location/activity data will be constructed. This work will enable the production of a computer subroutine that can be used to model total human exposure to environmental pollutants. The subroutine will generate human activity patterns and associated microenvironments necessary for exposure estimation. Also, insufficiencies in human activity pattern data will be identified during the proposed research. Such identification will lead to suitable recommendations for future human activity pattern research.

Identified Results: **Short term:** Identify if additional activity pattern data need to be collected and, if so, why.

Long term: Develop a valid data base for modeling exposure for the Agency's risk assessment efforts.

FY90 - report the results of the comparison of the 1985 - 86 National Activity Patterns Survey data and the 1987 - 88 California Activity Pattern Survey data.

FY91 - report the results of testing the hypothesis related to the influence air quality has on activity patterns.

FY92 - report results from studies of children's activity patterns related to dermal and inhalation exposure.

Usefulness of Results: Although past field studies have provided exposures for the population in a few U.S. cities, those data are insufficient to predict annual exposures of the U.S. population. To

extend the findings from these few cities to other parts of the country, activity patterns in these cities must be compared with activity patterns throughout the U.S. These analyses will allow us to generalize estimated exposures of the U.S. population for use in Agency risk assessments.

Project Length and Cost: 3 yr FY89: $150K

Project Officer: J. Beher (OMMSQA; EMSL-LV) (702) 798-2216

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Topic: Integrated Exposure Assessment

Issue: 2.2 Human Activity Patterns

Status: New

Title: Develop National Database of Activity Patterns for Exposure Assessment

Description: This project will design and collect a national activity pattern data base specific to exposure assessment. Although several large activity pattern data bases exist, none of these contain all the activity variables and information required for human exposure modeling. Use of representative probability sampling techniques will permit a new EPA activity pattern survey on a national scale that will provide the data needed to meet exposure modeling needs at a reasonable cost. Prior to conducting the large-scale survey, the researchers will conduct a pre-test survey to evaluate questionnaires and data collection techniques.

A small-scale pilot activity pattern field survey will be undertaken. Questionnaires and diaries will be evaluated using indepth interviews and focus groups. Respondent-following techniques will be tested to compare the quality of the data generated through questionnaires, diaries, interviews, and telephone recall methods. In addition, an automated diary datalogger will be evaluated as a possible technique for providing more accurate activity pattern information than traditional pencil-and-paper or recall methods. An automated sensor capable of monitoring the times spent in indoor and outdoor settings will be included in this pilot pre-test study.

This project will also formulate sampling protocols for a multi-stage probability sample of the U.S. population. The sample will be stratified to provide suitable urban and regional information for exposure modeling.

Identified Results: **Short term:** A standardized exposure-related survey instrument.

Long term: A national exposure-related data base for use in exposure modeling.

FY90 - results from pilot testing new exposure-related survey instrument.

FY91 - a survey of national activity patterns in the United States.

FY92 - results showing differences and similarities of activity patterns by geographic region, socioeconomic status, seasons, and other factors.

Usefulness of Results: One of the weakest components of human exposure models is inadequate human activity pattern data, particularly on a nationwide scale. The activity pattern information provided by the large-scale survey is "generic" in that it can support development of improved human exposure for several different pollutants and classes of pollutants. This research provides the basic tools required for conducting a large-scale activity pattern survey.

Project Length and Cost: 3 yr FY89: $150K

Project Officer: W. Nelson (OMMSQA; AREAL-ATP) (919) 541-3184

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Topic: Integrated Exposure Assessment

Issue: 2.3 Indirect Exposure

Status: Ongoing

Title: Pica Ingestion Rates in Children

Description: This project will examine the soil ingestion rates of children exhibiting pica behavior. In work conducted to date, EPA has studied soil ingestion in a population of one hundred children in the state of Washington (final report completed). Additionally, a follow-up field study with a subgroup of these same children was conducted to determine how measured ingestion rates vary over a longer time period and to make a first evaluation of soil ingestion in adult subjects (parents of the participating children). At the same time, a study carried out by the University of Massachusetts, earlier work from the Centers for Disease Control, and research conducted in the Netherlands contribute to our understanding of ingestion rates in "normal" children. However, a question regarded as critical in many EPA assessments, the rate of soil ingestion by children having "pica" behavior, is not addressed directly in this research. This project will support an examination of soil ingestion in children exhibiting such behavior.

In planning to study pica children, the researchers found that the first major obstacle was the identification of a population of children who exhibit pica behavior (regular ingestion of non-food materials). Therefore, a first task is a study to determine the frequency of pica behavior (as observed by parents) in a population of children six years and under. This will be done through a survey of parents of young children in an area, or areas, to be selected. Parents will be asked to report observed mouthing, soil ingestion, or other pica behavior using a graded frequency scale. These data will allow an assessment of both the prevalence of pica behavior and its nature. Since no expert consensus exists on exactly what range of behaviors should be termed pica, this study will in effect develop a working definition of the problem. Among the subjects identified as exhibiting significant pica behavior, a subset will be selected, randomly, for measurement of soil ingestion.

Identified Results: **Short term:** In FY89 a cooperative agreement will be initiated with the Fred Hutchinson Cancer Research Center to conduct the field component of this research, establish field procedures, and develop a working definition of pica.

Long term: Report on prevalence of pica behavior in young children and soil ingested by these children.

FY90 - identification of a sub-population of children with pica for measurement of soil ingestion.

FY91 - report on prevalence of pica behavior in children in a selected metropolitan area.

FY92 - report on the effect of the prevalence of pica on the sample weights used in risk calculations.

Usefulness of Results: Estimates of soil ingestion are necessary for the majority of agency assessments addressing contaminated sites and surfaces. Existing research has made much progress toward identifying a typical or average level of soil ingestion by young children. However, estimates of ingestion by children exhibiting pica are needed to improve the basis for individual "reasonable worst case assessments." Additionally determining the size of the actual pica population has much importance in the weighing of calculated risks. This project will efficiently address these questions. EAG is aware of no other research that will provide these data.

Project Length and Cost: 3 yr FY89: $125K

Project Officer: P. White (OHEA-EAG) (202) 382-2589

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Topic: Integrated Exposure Assessment

Issue: 2.3 Indirect Exposure

Status: New

Title: Selection of Food Consumption Rates

Description: This project is designed to improve the accuracy of the food consumption rates used in current Agency risk assessments. Food consumption data play a major role in many Agency exposure and risk assessments. Assumptions about the quantity of contaminated fish consumed can strongly influence risk estimates from water pollutants. Contaminated surface sites or leaking landfills can in turn contaminate local water bodies and thus risk assessments become dependent on fish consumption rates. Animals grazing or vegetables raised in contaminated soils may be consumed locally or distributed commercially, with exposures dependent on levels of consumption of these foods. The process of setting tolerances for pesticide residues involves selecting consumption rates for the particular crops.

Over the years different EPA offices have developed varied food consumption estimates to deal with these and related problems. However, the agency has not conducted a systematic examination of the methodologies appropriate for deriving these estimates. A number of large-scale studies have examined food consumption patterns across the U.S. population; however, application of these study results to EPA needs can be problematic.

A particular difficulty is that food consumption data are most commonly obtained for short periods of time with, for example, individuals reporting their intakes of food over a three-day period. EPA's needs, on the other hand, are for long-term or "usual" consumption data: Individual risk estimates are dependent on assumed lifetime average food consumption rates.

In this project we will build on existing research being carried out by other federal agencies to develop a recommended strategy for estimating rates of consumption of foods and apply this strategy to form estimates of consumption rates for a small number of sample foods. Food survey experts at the U.S. Department of Agriculture are currently sponsoring basic statistical research to determine methodologies for estimating usual consumption patterns. Similarly, the National Academy of Sciences and the Federation of American Societies for Experimental Biology have sponsored recent fundamental research on means of obtaining usual food consumption rates. On initial examination, the results of this research are encouraging. In this project we will select the most promising of the statistical procedures developed, make appropriate modifications, and address the long-term consumption rates for foods of particular EPA interest. Much existing research is oriented toward evaluating human nutrition, and alternate approaches may be required for evaluating foods consumed with low frequency.

A particular issue to be examined is fish consumption rates. Work is underway to compile recent data on fish consumption rates, which will form the starting point for improving fish consumption estimates. EAG is cooperating with the USDA in developing questions for a planned survey of national food consumption patterns. This work may yield an added bonus for this project by providing a means to define "regular consumers" of particular food products, allowing survey data to be separately broken out for these groups. In this case, some of the analytical effort in this project will focus on evaluating these results.

Identified Results:

Short term: Report on appropriate methodology for estimating accurately long-term food consumption rates.

Long term: Improved estimates of food consumption rates used in Agency risk assessments.

FY90 - report on statistical methodology for addressing EPA needs for long term food consumption data.

FY91 - recommended methodology of assessing fish and seafood consumption rates.

FY92 - report on modifications to short surveys for improving the surveys' ability to identify regular consumers of food products.

Usefulness of Results:

As indicated above, food consumption rates are central to many important risk assessments conducted by EPA. In some cases large uncertainties in intake rates are important factors in the uncertainty in the bottomline risk estimate. For example, fish consumption rates ranging from a few grams per day to a few hundred grams have been used in assessments for contaminated water bodies. This research will lay the groundwork for developing methodologies by which reliable and appropriate consumption estimates can be made. In addition, explicit analysis of data on fish consumption and consumption of other selected food will aid immediate Agency risk assessment needs.

Project Length and Cost: 2 yr FY90: $100K

Project Officer: P. White (OHEA-EAG) (202) 382-2589

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Topic: Integrated Exposure Assessment

Issue: 2.3 Indirect Exposure

Status: New

Title: Measurements of Exposures to House Dust and Soil Ingestion

Description: The long-term goal of this project is to accurately measure the contribution of house dust and soil ingestion to children's exposures to pesticides and metals. The amount of household dust and soil ingested by toddlers may be a major contributor to their exposure to certain metals and pesticides. Little is known concerning either the amount that they ingest or the levels of metals and pesticides in household dust. One reason is the absence of reproducible sampling methods for household dust. Recent work by EMSL-RTP (now AREAL) has resulted in the development of a sampler that collects reproducible amounts of dust while preserving the integrity of the sample for later chemical analysis. These samples will be used in a study—under controlled conditions—of several homes selected to represent a range of possible environmental conditions. Children will be observed during their household activities and areas where they play will be sampled before and after play. Amounts of dust and soil on hands will also be measured and analyzed for elemental and chemical content.

Identified Results: **Short term:** Report on construction and monitoring protocol for deployment of a house dust sampler.

Long term: Report of measurements obtained in a pilot test of the methodology. (Further long term research will not be funded.)

FY90 - report on the measurement of pesticides in house dust.

Usefulness of Results: The reports will be incorporated into scenarios of total human exposure, particularly the exposure of children, through air, water, food, and dust ingestion for those pollutants (metals, pesticides) that have several different pathways of exposure.

Project Length and Cost: 1 yr FY89: $150K

Project Officer: A. Bond (OMMSQA; AREAL-ATP) (919) 541-4329

Topic: Integrated Exposure Assessment

Issue: 2.3 Indirect Exposure

Status: New

Title: Short-Term Peak Exposure

Description: This project is designed to develop an estimate of short-term peak volatile organic compound (VOC) exposure. Several studies have indicated that short-term peak exposures to many pollutants may be extremely important contributors to total exposure. For example, a single hour spent stripping paint has been calculated to result in exposure to as much methylene chloride as a lifetime exposure to ambient levels. Clearly, such extreme exposures need to be accounted for in any realistic risk assessments.

Therefore, this project is designed to measure peak short-term exposures to a variety of pollutants in the most important microenvironmental situations. Emphasis will be placed on the most common microenvironments (measured in terms of millions or even hundreds of millions of persons visiting those microenvironments per year), and on the highest expected peak exposures, in order to obtain maximum efficiency.

The first pollutants to be studied will be a set of VOCs, including chloroform, other chlorinated compounds, and aromatic and aliphatic hydrocarbons. Crucial microenvironments for these substances are the shower (50% of a person's inhalation exposure to chloroform occurs during the 10 minutes in the shower;McKone, 1987); and use of household products (e.g., spray cleaners, air fresheners). Each of these activities will be studied in a careful quantitative way to allow much more precise estimation of exposures.

Identified Results: **Short term:** Based on available data, estimate the short-term peak to mean ratio for the VOCs measured in TEAM studies. Long- term research in this area will not be funded.

FY90 - report on results on the use of new short-term methods in measuring peak exposures and peak levels observed in selected microenvironments.

Usefulness of Results: These studies will provide data for use in risk assessments. These data can be input into models based on activity patterns, time budgets, and microenvironmental concentrations to estimate the proportion of total exposure contributed by the given microenvironment under different exposure scenarios.

Project Length and Cost: 1 yr FY89: $125K

Project Officer: G. Evans (OMMSQA; AREAL-RTP) (919) 541-3124

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Topic: Integrated Exposure Assessment

Issue: 2.3 Indirect Exposure

Status: New

Title: Measuring Contribution of Exposure from Attached Garages

Description: This project is designed to develop accurate estimates of the source contribution originating from attached garages emitting into the indoor residential microenvironment. The importance of the attached garage as a route of exposure to a number of toxic and carcinogenic compounds has only recently been understood. Many people store gasoline, kerosene, cleaning products, paints, pesticides, lawn mowers, snow blowers, and other potential emitters of noxious vapors in their attached garages, in addition to the automobile itself. A recent study of benzene exposure has identified the attached garage as a possible source of the elevated concentrations observed in homes.

An attached garage will be stocked with the products under investigation and measurements will be taken both in the garage and in the living quarters to determine the impact of each source. Measurements will be made with canisters for VOCs and PUF cartridges for pesticides. Qualitative and quantitative identifications of a large number of compounds will be made to apportion sources using established "signatures" for each major source category. (Initial work to establish such signatures will be done for those source categories for which information is inadequate.) Air exchange between garage and home, as well as between the garage and the outdoors and the home and the outdoors, will be carried out using the multiple-tracer PFT technique developed at Brookhaven. Actual emission rates of some or all of the major source categories will be measured in headspace or chamber studies, to compare with calculated emission rates using the air exchange information. Studies of effect of evaporative emissions will also be undertaken to estimate the contribution of the car to overall exposure levels.

Identified Results: **Short term:** (FY90) a report of the relationship between emission rates, garage-home air exchange rates, and human exposure will be established for a variety of garage scenarios.

Usefulness of Results: This project will provide realistic estimates of the impact of an attached garage on indoor air quality levels. In turn, these estimates will improve total human exposure estimates for use in Agency risk assessments.

Project Length and Cost: 1 yr FY89: $75K

Project Officer: R. Highsmith (OMMSQA; AREAL-RTP) (919) 541-7828

APPENDIX C
TOPIC 3: PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS PROJECT DESCRIPTIONS

Note: No description is provided here for Project 3.1.1(6) because it is a support, not a research, task.

Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.1 Experimental Absorption and Biological Parameter Data

Status: Ongoing

Title: Experimental Studies for Models of Dermal Absorption of Chemicals

Description: The goal of this project is to improve the accuracy of predictions of dermal absorption and disposition for chemicals of interest to the Agency. Parallel experimental studies will be conducted in animals and in humans to examine and evaluate models for predicting human skin penetration.

Any model—whether animal or mathematical—intended for use in a regulatory setting must have a sound biological foundation. This study therefore 1) examines the relationship between factors such as chemical structure and skin absorption and 2) compares the output from existing models for quantifying the dermal penetration with data gathered from human volunteers. The models examined include:

- model skin lipid membranes (rotating diffusion cell)
- in vitro methods using mouse and rat skin
- rat in vivo model
- human skin grafted athymic nude mouse model

Initially, a series of para-substituted phenolic compound will be used that were selected as part of a Cooperative Agreement with the University of San California at San Francisco. These will be used to examine the relationship between chemical structure and skin absorption, including the substituents for hydrophobic (Hansch), electrostatic (Hammett), steric (Taft E), dispersive (molar refractivity), and dermal penetration.

One of the critical parameters involved in the assessment of risk is the potential for exposure of the target population to the agent(s) being evaluated. The dermal route of exposure is the major pathway for many environmental compounds, especially pesticides. At present, there are no generally accepted laboratory models for the determination of potential human dermal absorption. Proposed methods include quantitative structure activity relationship (QSAR) models (Project 3.3.2(1)), in vivo animal models (using a variety of animals species), and in vitro systems that rely on both viable and nonviable animal and human skin. The usefulness of these models for extrapolating human exposure levels has been questioned because of:

- the role of large interspecies differences in skin structure
- the lack of relevant metabolic capabilities in the in vitro methods

This project has been designed to collect data using the most promising methods for assessing skin absorption so that the accuracy with which the various approaches predict human response can be evaluated. In addition, the usefulness of the QSAR approach and

other factors in predicting dermal penetration and improving understanding of the mechanisms of skin transport will be examined.

Identified Results: **Short term:** Evaluation of methods to predict skin penetration in humans

Long term: Assessment of QSAR approach for predicting dermal penetration of phenols and other classes of chemicals; identification of factors (e.g., dose, chemical form) associated with predicting dermal ingress.

These results can be used by all Program Offices in the Agency that are involved in risk assessment to increase the precision of target tissue dosage estimation.

FY89 - reported results on the model lipid membrane system with phenols, the in vitro mouse skin studies, and the solid state studies

FY90 - reported results on the dermal penetration of the substituted phenols in rats

FY91 - reported results on the high-molecular-weight studies and on the dermal absorption of phenols in humans

FY92 - reported results on the dermal ingress of phenols in the human skin grafted athymic nude mouse model

Usefulness of Results: Hazard evaluation is often made on the basis of structure-activity relationships. This research effort is exploring biological and mathematical approaches for predicting percutaneous penetration on the basis of chemical structure and properties as well as generating a database that can be used in constructing PB-PK models for the phenols. This task links with 3.3.1(2) by providing pharmacokinetics that will assist in interpreting the embryonic dosimetry results.

Project Length and Cost: 3 yr FY89: $330K

Project Officer: L. Hall (OHR/HERL) (919) 541-2774

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.1 Experimental Absorption and Biological Parameter Data

Status: Ongoing

Title: Experimental Deposition in Man of Inhaled Insoluble Particles for Development and Validation of PB-PK Models

Description: This project will provide a comprehensive database on the deposition and fate of inhaled particles in humans. The output of this database will feed into pharmacokinetic models of inhaled particles (Project 3.3.1(2)), which are intended to provide quantitative predictions of the dose of toxic particles to the lungs and other organ systems in humans.

The three primary components of the study are:

- Experimental determination of lung mucociliary clearance of inhaled insoluble particles in human volunteers
- Experimental determination of alveolar clearance of inhaled insoluble particles in human volunteers
- In vitro studies of the dissolution rates of toxic particles

Efforts under the first component will examine the patterns of mucociliary clearance of inhaled particles as a function of particle diameter in a population of healthy human volunteers. Mucociliary clearance of inhaled particles occurs within the first day following an acute particulate inhalation. The data gathered will help explain particle retention as a function of time during the first hours following exposure. The retention of particles 24 hours after inhalation will also be measured; particles retained for that length of time are deposited in alveolar regions of the lungs.

The particles the subjects will inhale will be either ferric oxide or teflon particles labelled with the radioisotope Tc-99m. Following inhalation, a gamma camera that provides a two-dimensional representation of the activity distribution will be used to measure the radioactivity retained in the lungs. The initial particle deposition distribution and clearance patterns can thus be measured simultaneously.

Activity in the head and stomach will also be measured as a function of time to quantitate translocation of particles from the lungs and extrathoracic airways to the stomach. The technology required for this work has already is already available at the Clinical Research Branch. Transport rates of mucus within individual airways will be assessed by placing isolated quantities of particles within individual airways either through introduction of a fiberoptic bronchoscope or by inhalation of aerosol boluses. The latter is the more desirable method because it is noninvasive; however, the results need to be confirmed.

Work on the second component of this task will examine the slower patterns of alveolar clearance of inhaled particles as a function of particle diameter in a population of healthy human volunteers. Longer-lived radioisotopes such as In-111 will be used because particle retention will be measured over a period of several days rather than several hours. More sensitive radiation detectors will therefore be required because smaller amounts of initial activity will be used.

Activities associated with the third component of this study will determine the rates of dissolution and release of toxic compounds that adhere to toxic particle surfaces. These studies will be conducted in vitro utilizing fluids that are either obtained directly from human volunteers by bronchoalveolar lavage, or manufactured and tested for biocompatibility with bronchoalveolar lavage fluid. In vivo studies may also be used to measure the disappearance rates of soluble particles and to determine the remaining fractions of compounds in particle and dissolved states. This last component of the study will provide the final link in the chain of events between inhalation of particles and release of constituent compounds within the lungs.

Identified Results: **4th Q/89** - Interim results from aerosol deposition and mucociliary clearance study and from the particle dissolution study. These data will provide important information to program offices on establishing chronic doses to tracheobronchial epithelium from inhaled particles of various solubilities. They will also comprise important input into the

pharmacokinetic modeling project (3.3.1(2)), which will enable accurate predictive dosimetry.

FY90 - Final data from aerosol deposition and mucociliary clearance study and from the particle dissolution study. Interim data on alveolar deposition and clearance of particles. The data, even though available only for a limited range of particle sizes, will provide program offices with vital information on the residence times of inhaled particles in the gas exchange regions of the lungs—the site of high chronic particle lung burdens. This information will thus lead to better estimates of the dose of chronically inhaled particles. Also, the data will be used as input into the pharmacokinetic modeling project (3.3.1(2)) to further enhance the accuracy of predictive dosimetry.

FY91 - Final data on the deposition and alveolar clearance of inhaled particles over a complete range of particle sizes. Both directly, and through the pharmacokinetic modeling, these data will enable program offices to predict the dose of inhaled particles to various regions of the respiratory tract as a function of particle size, breathing pattern, route of inhalation, and particle solubility.

Usefulness of Results: For a known ambient particle concentration of a toxic aerosol, our ability to predict the potential toxic effects in humans depends on our knowledge of the initial distribution and subsequent fate of these particles within the lungs. The initial distribution of inhaled particles is highly dependent on their size and the pattern of breathing. The fate of deposited particles depends on the region of the lungs in which they are deposited and on the physicochemical properties of the particles. Particles deposited in the conducting airways of the lungs are transported cephalad toward the larynx by mucociliary transport, at which point they are swallowed. Particles deposited in the gas exchange region of the lungs are cleared from this region by a variety of processes. Particles can be engulfed by macrophages and translocated either to the ciliated airways of the lungs or into interstitial spaces. Pinocytosis by alveolar epithelial cells is also possible, as is direct translocation of free particles either to the ciliated airways or into the interstitium and blood. Overlaying all of these processes is the mechanism of particle dissolution, whereby the particles decompose into their chemical constituents. The residence times of particles and their chemical constituents in different regions of the lungs—and the rate of their delivery to other organ systems—depend on the detailed kinetics of the particle transport and clearance mechanisms. Although some experimental information has been gathered on particle clearance mechanisms, it is not sufficient for providing accurate quantitative dosimetric information. Data resulting from this project will include particle loads within respiratory tract compartments as a function of particle and breathing parameters, as well as clearance rate constants for these compartments. Particle dissolution is also included as a clearance compartment. These data will form the basis of modeling the chronic retained dose of soluble and insoluble particles. The models will provide predictive dose information on compounds in particulate form and thereby improve heath risk assessment.

Project Length and Cost: 3 yr FY89: $130K

Project Officer: T. Gerrity (OHR/HERL) (919) 541-2567

Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.1 Experimental Absorption and Biological Parameter Data

Status: Ongoing

Title: Dosimetry of Hygroscopic Aerosols in Humans

Description: This project will provide data on the respiratory tract deposition of hygroscopic aerosols in spontaneously breathing humans. This information will then be used to validate models of hygroscopic aerosol deposition—which can, in turn, be applied to animal species and thus serve as an extrapolation link for acid aerosol dose between animals and humans.

Respiratory tract deposition of insoluble aerosols in humans is measured by comparing the particle number concentration between inspiration and expiration. The instrumentation required to accomplish these measurements requires that the particle size between inspiration and expiration not change. For insoluble aerosols, this restriction does not pose a problem. Hygroscopic aerosols, however, can alter in size as they traverse the humid environment of the respiratory tract. Standard methodologies for measuring particle deposition in the respiratory tract cannot therefore be applied. A new technique of aerosol characterization that simultaneously—and in real time—measures particle size and concentration is currently under development at the University of Arkansas under funding from EPA. This methodology should be applied to the measurement of hygroscopic aerosol particle deposition in the human respiratory tract. Emphasis will be on acid aerosols because these compounds are ubiquitous in urban air and are of immediate regulatory interest.

This research effort will be multistaged. The first set of experiments will measure the deposition of aerosols, such as sulfuric acid, in humans inhaling through a mouthpiece. The deposition studies will examine factors affecting deposition: tidal volume, ventilatory flow rate, temperature and RH of the aerosol, and acid species. These initial studies will help to test and refine models of acid aerosol deposition currently under development at HERL.

The second set of experiments will measure the deposition of hygroscopic aerosols under conditions that better simulate natural spontaneous breathing. This phase requires the development of an aerosol delivery system that will allow subjects to breathe orally, nasally, or oronasally, and that will simultaneously measure the inspired and expired concentrations of aerosol. These data, which will be of more direct regulatory significance because the breathing conditions will be more natural, will provide a picture of the population variability of deposition.

In a third set of experiments, the subjects will be exposed to hygroscopic aerosols in a chamber so as to induce acute responses such as retardation of mucociliary transport and, possibly, bronchoconstriction (in asthmatics only). Hygroscopic aerosol deposition in these subjects will be measured simultaneously so that a relationship between delivered dose and response can be determined.

Identified Results: **4th Q/89** - Data on the deposition of sulfuric acid aerosol in humans as a function of flow, tidal volume, temperature, and RH during mouthpiece breathing. These data will allow direct computations of sulfuric acid doses to respiratory tract epithelium under a variety of ambient conditions that could affect particle characteristics.

FY90 - Data on the deposition, by mouthpiece, of acid aerosols as a function of acid species. Data on the respiratory tract deposition of acid aerosols by spontaneously breathing humans inhaling the aerosol orally, nasally, or oronasally. This information will facilitate better quantitation of delivered acid aerosol doses based on more physiologically normal conditions for breathing.

FY91 - Data on the relationship between deposited acid aerosol dose and acute health effects. These data will help the Office of Air Quality Planning and Standards to assess the variability of delivered dose and of response in the population at large; as a result, better predictions will be made of the population impact of acid aerosol health effects.

Usefulness of Results: Experiments in humans have shown that exposure to low concentrations of sulfuric acid aerosols can produce acute retardation of mucociliary transport in the periphery of the lungs. Low concentrations of sulfuric aerosol may also induce bronchoconstriction in persons with asthma. Animal studies have shown similar results with respect to retardation of mucociliary transport; in addition, prolonged exposure has been shown to permanently depress mucociliary transport. Because such transport of inhaled particles is an important lung defense mechanism, this depression could have significant health implications. To extrapolate the chronic animal study data to humans, estimates must be made of the dose of acid aerosol delivered to lung tissue in both humans and animals.

Little is known of the dosimetry of hygroscopic aerosols in unencumbered, spontaneously breathing humans. One reason for the paucity of data is the aqueous nature of the aerosols: during respiration, they change in size and thus the manner in which these particles are distributed within the different compartments in the lungs also changes. Recent technological advances in aerosol measurement now allow simultaneous measurements of particle size and number, so that aqueous aerosol deposition can now be studied. Data from this project will be of extreme interest to the Office of Air Quality Planning and Standards for estimating the risk associated with acid aerosol exposure in humans. These data will allow the extrapolation of data from chronic acid aerosol exposure in animals to humans. Moreover, advancements in understanding the deposition of hygroscopic particles will be of interest to other Program Offices as well.

Project Length and Cost: 3 yr FY89: $100K

Project Officer: T. Gerrity (OHR/HERL) (919) 541-2567

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.1 Experimental Absorption and Biological Parameter Data

Status: Ongoing

Project Code: 3.1.1(5)

Title: Permeation Coefficients and Comparison of Dermal Absorption Models

Description: PB-PK models that account for dermal absorption by two different approaches. First, sufficient data and modeling may be available for the compound after exposure by another

route. In this case, a forcing function is written as the mathematical description characterizing the blood concentration after dermal absorption. This approach would require laboratory experimentation to determine the blood levels after dermal exposure. The necessary parameters, however, are easily determined. Models derived in this manner would be useful to risk assessors attempting to estimate delivered or target dose in animals for the dermal exposure situation when the bioassay experiments were performed by another route. The potential difficulty in extrapolating from one species to another and, in some cases, from one dose to another is the primary disadvantage of this technique.

A second and more global approach is to write PB-PK models that describe the physiology and thermodynamics of the dermal absorption process. Even for the simplest of models, the key required parameter is the permeation coefficient across the skin. Although numerous reports have described methods for determining this parameter, but several questions remain. It is not clear, for example, which in vitro model is best for determining the in vivo permeation coefficient. Nor is it apparent whether animal or human skin is best suited for determining these coefficients. Further, if animal skin is chosen, which skin is a good surrogate for the human condition? Or, if human skin is used, from what region of the body should it be taken, or should cultured skin cells be used instead? Once a proper method is chosen, these coefficients can be accurately determined and then used in PB-PK models. A framework is necessary that outlines the appropriate method for determining the necessary parameters for given conditions and available data.

Several compounds with varying physical properties will be chosen. PB-PK models describing transfer across the skin and deposition in the body will be written for these compounds. The literature will be searched for pharmacokinetic data that can be used to validate and refine the pharmacokinetic models. Following model formulation and examination of existing data, the experiments necessary for filling data gaps will be identified and the appropriate protocols written. On-going research involves reviewing existing literature and consulting with experts regarding the various in vitro methods of determining permeation. From this research, a framework will be prepared that indicates the best methods for determining permeation coefficients for specific compounds, available data, and exposure conditions.

Identified Results: Several PB-PK models for compounds that can potentially be absorbed through the skin. A framework for using such models to predict the extent and rate of dermal absorption and the disposition to target organs after dermal absorption. A synopsis of the various methods used for determining transdermal permeation and a hierarchy for choosing the best method.

Usefulness of Results: Describing and predicting the degree of dermal absorption of xenobiotics using current methodologies is difficult and results in uncertain estimates. It is not clear which laboratory procedures for either whole animal or in vitro testing are applicable under which conditions. Further, even with laboratory determinations, it is not clear how to extrapolate to human exposure conditions. In recent years, PB-PK models have been used with success to predict disposition of xenobiotics in the body and at potential target sites within the body. The key parameter in models predicting disposition after dermal absorption is the permeation coefficient. This study will provide methodologies for the best and most efficient manner of determining permeation coefficients and formulating and implementing PB-PK models.

Project Length and Cost: 3 yr FY89: $50K

Project Officer: K. Hoang (OHEA-EAG) (202) 382-2059

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.1 Experimental Absorption and Biological Parameter Data

Status: New

Title: PB-PK Data and Models for Compounds with Known Multispecies Carcinogenic Responses

Description: Recently, through an Interagency Agreement, OHEA and the Department of Defense reviewed exposure and carcinogenesis data for about twenty compounds known to be carcinogenic in more than one species, including humans. The goal is to determine if animal-based risk estimates, as they are now performed, are within reasonable limits of risk estimates based on human data. These data were also used to determine the best dose metric for extrapolating risk to humans from estimates derived in animals. Often the data were taken from a variety of exposure scenarios for both animals and humans. Thus, establishing comparability of exposures is difficult. Establishing an equivalent dose metric among species requires knowledge of the actual target tissue dose after the various exposure regimens. With those data, exposures at very different concentrations and with different regimens could be equated, based on the resultant internal doses. PB-PK models offer an excellent opportunity to accomplish this task. For most compounds, however, adequate models have not yet been developed.

Representative compounds will be chosen for formulation of PB-PK models. After a review of the pharmacokinetic data and formulating models for the compounds, any data gaps for the compounds (e.g., partition coefficients or metabolic rate constants) will be identified. Those compounds with the most complete pharmacokinetic data will then be chosen for complete study. The models formulated and validated for these compounds will be used to calculate the actual target dose in the various species for which carcinogenic responses occur. With these data, the best dose metric can be chosen for each compound. At that point, efforts using both inhouse and extramural resources can begin to resolve the problem of equivalent dose metric across species. Outside resources will concentrate on reviewing and obtaining the necessary data, while inhouse staff will formulate and implement the PB-PK models and apply the results to the question of dose equivalency. Necessary laboratory experiments will be performed either inhouse or by outside resources.

Identified Results: A review and collation of the available pharmacokinetic data for several human and animal carcinogens. PB-PK models for some compounds. These models can be used to determine the impact of different exposure regimens on risk. As a result, the importance of pharmacokinetic nonlinearities—which may not be identical at different exposure levels and regimens—can be accounted for in future site-specific risk assessments. Ultimately, this project would begin to resolve the question of equivalent dose metrics between species and would also provide a design for future study of this problem, even with endpoints other than carcinogenesis.

Usefulness of Results: Perhaps the greatest controversy concerning risk assessment techniques involves the uncertainty with which risk assessors extrapolate between species. No method for equilibrating dose between the test species and a species at potential risk (usually the human) has ever been universally accepted. The problem is particularly difficult because, even in those few cases in which response data exist, the actual dose to the target tissue is not known. Another complicating factor is the lack of equivalence between the exposure regimens for each of the tested species and the species at risk. This project will forge a reliable methodology for determining target tissue doses under the various exposure conditions, thus allowing comparison of response vis-a-vis dose across a myriad of species.

Project Length and Cost: 3 yr FY90: $75K

Project Officer: P. White (OHEA-EAG) (202) 382-2589

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.1 Experimental Absorption and Biological Parameter Data

Status: Ongoing

Title: Pharmacokinetics of Inhaled and Ingested Volatile Organic Compounds

Description: The objective of this project is to develop and validate PB-PK models intended to accurately predict the concentration of halocarbons in blood and tissues over time following oral or inhalation exposure. The models can be used in dose-to-dose, route-to-route, and species-to-species extrapolations; thus, they can be used to estimate the internal organ/tissue exposure from a known or hypothesized external exposure scenario for humans. The health effects of this internal exposure can then be estimated using the human organ/tissue dose-response relationship.

PB-PK models have been developed and validated on the basis of results from:

- Intravenous injection
- Inhalation exposure (at two vapor concentrations)
- Oral bolus
- Gastric infusion (at doses equivalent to inhalation exposure)
- Multiple oral doses

With these methods and results as a basis, studies will be performed to delineate the time course of tissue levels in rats during pulmonary and oral exposure to several different halocarbons at equivalent doses. Empirical measurements will be made of important physiological parameters, e.g., in vivo tissue-blood partition coefficients, clearance values, metabolic constants, and tissue volumes (especially in adipose tissue). PB-PK models for inhaled or ingested halocarbons will be developed, validated, and modified on the basis of the database obtained. Validation of the models will be done with low-level exposures, including simultaneous oral and pulmonary exposure. Once the models have been validated, the data will be extrapolated to human exposures, and the results compared with existing data on the chosen compounds.

Identified Results: A large pharmacokinetic database, consisting of inhalation-and-ingestion absorption and distribution data for several volatile organic compounds. Validated pharmacokinetic models, based on both oral and pulmonary exposure, together with their extrapolations to humans and comparison with human data. A set of measured physiological parameters important in PB-PK modeling. Finally, a framework for developing a complete pharmacokinetic approach that incorporates both ingestion and inhalation exposures.

Usefulness of Results: The validated PB-PK models produced will be used as input into biologically based dose-response (BB-DR) models and thus for health risk assessments. The models can be used in high-to-low-dose, route-to-route, and species-to-species extrapolations, and thus to estimate the internal organ/tissue exposure form a known or hypothesized external exposure scenario for humans. The health effects of this internal exposure are then estimated using the human organ/tissue dose-response relationship, which may be based on animal experimental data, animal PB-PK models, and human-animal relative sensitivity from (often) tissue and cell culture experiments. Validated PB-PK models enable the extrapolation of experimental results from animals in laboratory controlled environments to humans in ambient uncontrolled environments, thereby reducing the uncertainty in health risk assessments.

Project Length and Cost: 3 yr FY89: $117K

Project Officer: T. Gerrity (OHR/HERL-RTP) (919) 541-2567

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.1 Experimental Absorption and Biological Parameter Data

Status: New

Title: Database Synthesis of Existing Biological Parameters

Description: This project uses available expertise to develop consensus opinions on the values of certain physiologic parameters commonly used in PB-PK models. As a result of an Interagency Agreement, staff at Oak Ridge National Laboratories have summarized literature values for physiological parameters such as organ volumes, blood flows, and body weights for several mammalian species. The parameters chosen generally fit into the simpler PB-PK models. This project funds the EPA portion of an Interagency Agreement with the Food and Drug Administration (FDA) and the Department of Defense (DOD) to empanel experts selected by the American Physiological Association to review the Oak Ridge work, expand the chosen parameters, and arrive at a consensus of ranges for the values of the parameters. The panel, which will be composed of national experts in physiology, will issue a report.

Identified Results: Report detailing range of values for physiologic parameters such as blood flow and organ volume in several mammalian species that are commonly used in toxicological testing.

Usefulness of Results: Having standard, peer-reviewed values for physiologic parameters used in PB-PK modeling would greatly improve confidence in the models. The goal of pharmacokinetic modeling is the reduction of the uncertainties inherent in risk assessment. The models,

however, have their own intrinsic uncertainties. Accurately establishing as many of the model parameters as possible is vital to the models' usefulness. Some parameters are case-specific, while others (such as the physiologic parameters, have ranges that can be narrowed significantly. To date, examination of the literature has revealed no consensus is available on appropriate ranges for these parameters. This project will establish such a consensus, thus reducing the model variation that results from the arbitrary selection of parameter values.

Project Length and Cost: 1 yr FY90: $15K

Project Officer: W. Farland (OHEA) (202) 382-7315

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.2 Route-to-Route Extrapolation

Status: New

Title: Guidance on Limitations and Use of Route-to-Route and Other Extrapolations in EPA Risk Assessments

Description: PB-PK models have become useful tools for estimating target-tissue doses and thus represent a critical advance in risk assessment methodology. Based on the premise that a particular target-tissue dose will have the same biological effect as an equivalent target-tissue dose achieved by another exposure regimen, these models may be used to predict delivered doses under conditions of exposure different from those initially tested, including different:

- Routes of administration
- Exposure concentrations
- Time patterns of exposure

Risk assessment extrapolations such as these require equations and biologic parameters sufficiently detailed to describe the differences in absorption by various routes and to account for the structural and physiologic differences between species.

Panels of experts will be commissioned to:

- Discuss the critical parameters, assumptions, and limitations related to route-to-route extrapolation
- Provide specific guidance on the use of PB-PK models and associated algorithms in risk assessments
- Recommend research areas in which research efforts would facilitate future extrapolations

The research recommendations and guidance arising from this exercise will be incorporated into ongoing ORD research programs and risk assessment methodologies.

Identified Results: Guidance in the form of a report on the use of PB-PK models and algorithms, incorporating key parameters identified by consensus. Recommendations on research areas that will facilitate the various extrapolations required in risk assessment (e.g., route-to-route, high-to-low exposure concentrations, and intermittent dosing regimens to chronic scenarios) for use in focusing ORD research programs on filling critical data gaps.

Usefulness of Results: Databases used in risk assessments often lack sufficient toxicological and pharmacokinetic information for the route, dose rate, and regimen of exposure relevant to the human exposure scenario. Consequently, current methodologies use various assumptions and default values to extrapolate to the desired situation using other available data. These approaches neither systematically incorporate known anatomic and physiologic interspecies differences in the routes nor utilize pharmacokinetic or pharmacodynamic properties of the agent and species in question. In addition, the approach for such extrapolations often differs across Program Offices. Improved approaches are needed that model the absorption/distribution/retention processes in sufficient detail to estimate the target-tissue dose for various exposure scenarios. As a result of such work, the Agency will not have to request extensive, time-consuming, and often expensive disposition studies for each agent and scenario of interest.

This project will provide expert scientific input to achieve consensus on the criteria, assumptions, and key parameters and limitations of application that are requisite for performing route-to-route or other extrapolations in risk assessments. Guidance in the form of a state-of-the-art report that explicitly defines limitations on application will ensure consistent use across Program Offices. Recommendations on research that would facilitate such extrapolations and strengthen the existing assumptions or eliminate default values will help to focus ORD research programs and ensure that Agency risk assessment methodologies evolve with scientific credibility.

Project Length and Cost: 2 yr FY89: $75K

Project Officer: T. Gerrity (OHR/HERL-RTP) (919) 541-2567

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.3 Theoretical Models

Status: New

Title: Regional Retained Dose Ratios for Adjustment of Respiratory Tract Burdens Across Species and Use in Inhalation Reference Dose Methodology

Description: Empirical data and existing theoretical models will be systematically analyzed, with an emphasis on aerosol exposures, to determine the anatomic and physiologic parameters necessary for the quantitation of an inhaled dose. Development of a model that incorporates factors to account for the regional (e.g., extrathoracic, tracheobronchial, and pulmonary) deposition in the respiratory tract as well as the fate of the inhaled particles over time will be based on these parameters. Factors that will be incorporated, as feasible, to account for the fate of inhaled relatively insoluble particles include:

- Mucociliary transport and clearance rates
- Alveolar clearance rate (phagocytosis/translocation by macrophages/dissolution/free particle translocation)
- Particle solubility
- Chemical activity

Certain data gaps for individual species will be filled empirically by contract—for example, consistent methodological procedures will be used to determine surface areas and clearance rates of the respiratory regions to ensure compatible and precise estimates for model input across species.

Output of the proposed model will be estimates of retained doses in the various regions of the tract for a given species used experimentally. Calculation of the ratios of these regional retained doses between a given experimental species and humans provides a factor for scaling an aerosol concentration to which the animal was exposed for interspecies differences in retention of the inhaled dose; thus, it will then be possible to more accurately estimate the equivalent exposure concentration for humans. Specifications on how to apply these ratios as scaling factors (such as guidance on which ratio is appropriate for an observed toxicologic effect either locally in the lungs or systemically) and limitations on their application will be explicitly stated. The characterization of anatomic and physiologic parameters and the filling of critical data gaps across species, involved in the development of the aerosol model, will provide the basis for mass transport estimates needed to expand and refine existing gaseous uptake models. A gas and vapor model that accounts simultaneously for characteristics such as solubility, reactivity, and metabolic transformation will then be developed.

Identified Results:

Short-term - An analytical model for derivation of regional deposited dose ratios by which animal experimental exposure concentrations can be scaled to human equivalent conditions for accurate inhaled aerosol dose estimation used in calculation of reference doses. The model will be used to generate regional retained dose ratios in a tabular format as part of a support document to the Inhalation Reference Dose Methodology. The technical support document will also provide guidelines on the application and explanation of the limitations for the procedural use of these ratios in the RfD methodology.

Long-term - Sensitivity and interaction analyses of the model parameters versus time to provide insight for supporting guidance on the limitations of acute versus chronic extrapolations. Analysis of the effect of concentration on the parameters to assess high-to-low dose extrapolations. Similar model development for various gases according to characteristics (and their combinations) such as: solubility, reactivity, and metabolic transformation. (Because the majority of agents of interest to Program Offices are gases, development of this model is urgent.) Support documents for inhalation RfD methodologies giving guidelines on dose adjustment for gas and vapor agents.

Usefulness of Results:

The Agency's intention to use reference doses as the principal values in assessing risks for noncarcinogenic health effects critically depends on accurate extrapolation of inhaled doses from animals to humans. The following, in other words, are needed:

- Precise estimates of the retained dose received by an animal species in a given exposure concentration
- Accurate extrapolation of that animal dose to an exposure concentration that would result in an equivalent retained dose in humans

The modeling effort under this project will provide ratios of precise regional retained dose estimates in animals to those of humans for use in scaling experimental aerosol exposure

concentrations to human equivalent conditions. These ratios thus provide the required dose estimates and extrapolation necessary for the operational derivation of the reference dose for accurate risk assessment of inhaled aerosols. The modeling effort will initially focus on aerosol deposition and fate because vast amounts of data and many theoretical models currently exist due to past efforts to quantify radionuclide exposures. However, identification and characterization of the anatomic and physiologic parameters necessary to estimate deposited doses of aerosols will support similar model development for mass transport of gases and vapors to more precisely estimate the retained doses of these agents. Accurate estimation of an inhaled dose and knowledge of governing parameters is also necessary before any dose partitioning for analysis of mixtures can be accomplished, a procedure that will be needed for analysis of risk on a source category basis. Analysis of the interaction of these parameters over time will provide insight on the limitations for acute versus chronic applications, while analysis of how certain factors are affected by concentration (e.g., clearance rates) will provide guidance on high-to-low-dose extrapolations; both are crucial outstanding areas of uncertainty in risk assessment.

Project Length and Cost: 3 yr FY90-92: $225K total

Project Officer: T. Gerrity (OHR/HERL-RTP) (919) 541-2567

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.3 Theoretical Models

Status: Ongoing

Title: Development of PB-PK Models Following Inhalation, Ingestion, or Dermal Exposure to Toxic Compounds

Description: This project takes data output from experiments on uptake and clearance of particles that are inhaled, ingested, and dermally applied. A thorough review of current knowledge regarding pharmacokinetics of particles administered by any route will be conducted. Because mechanisms of action vary and often result in first-order linear, second-order linear, or nonlinear kinetics, no universally applicable PK-PB model can be constructed. Models for general classes of chemicals, however, can be developed that provide a framework for work on individual chemicals in the class. In addition, the number of compartments in the mathematical model is appropriately determined by the level of understanding of the biological processes involved, the intended regulatory applications and their societal impacts, and the extent to which the model can be validated. The scope of work of this project is intended to provide a personnel skill mix to extend the capability of theoretical PB-PK modeling to OHR research efforts involving oral, dermal, or inhalation exposure. Current capabilities are largely restricted to dosimetry of inhaled compounds.

PB-PK models will be developed for various experimental efforts, such as the para-substituted phenols from dermal studies (Project 3.1.1(1)) and the human inhalation of insoluble particles (3.1.1(3)). In addition, for key toxicologic studies that are part of the OHR base program, some project efforts will encompass the development of PB-PK

models; this will allow future OHR research programs to provide risk assessors with the appropriate dosimetry models for evaluating the importance of observed biologic effects.

Identified Results: PB-PK models for various exposure routes (e.g., dermal, oral, inhalation). The PB-PK modeling for deposition and clearance of insoluble particles will receive initial emphasis to link with the efforts on inhalation reference doses (Project 3.3.1(2)). Also, the modeling effort needed to link exposure-dose-developmental biologic effect (links to 3.1.1(1) and 3.3.3(1) will be enacted in FY89.

Usefulness of Results: The assessment of the potential health risk from toxic chemicals depends on animal toxicology studies establishing an organ dose-response relationship. The extrapolation from the results of these studies to the potential for health impacts in humans requires the ability to relate the human exposure conditions directly to organ doses. One of the most promising means by which such extrapolations can be made is through mathematical modeling of the pharmacokinetics of compounds that are delivered by the inhalation, oral, and dermal routes.

To have the predictive power necessary for providing answers to regulatory questions, these models must be firmly based on quantitative data. All of the projects under Topic 3 will provide databases to support mathematical modeling. Resulting data will permit predictions of ambient concentrations of particles and vapors to which humans are exposed and that would result in specific doses of the compounds to systemic organs.

Project Length and Cost: 3 yr FY89: $240K

Project Officer: T. Gerrity (OHR/HERL-RTP) (919) 541-2567

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.3 Theoretical Models

Status: Ongoing

Title: Development of Mathematical and Physiological Bases for Model Validation, Formulation, and Sensitivity Analyses

Description: Pharmacokinetic model formulation requires an understanding of physiology, thermodynamics, biochemistry, and the specific database in question. No clear guidance is available on how to properly formulate a model for specific conditions. For example, modelers will often structure a particular model in a certain manner because the available data cannot be understood in any other way. This project will accomplish several tasks:

- Form a summary of pharmacokinetic models used to describe deposition and discuss the criteria used for selecting or formulating each model (summaries gathered by discussion with experts, literature review, inhouse implementation of existing models, and peer review of reports)
- Formulate guidance on what information must be gathered and what questions must be answered to formulate a PB-PK model

- Provide guidance for model validation
- Develop a decision tree approach to give guidance on model formulation in specific cases (this type of decision analysis lends itself to expert system automation)

During the first year, work will begin on just a portion of the PB-PK model formulation. In vivo metabolic rate constants are often case-specific and difficult to extrapolate between species. In vitro generated metabolic rate constants offer promise of ease of experimentation and the ability to use human tissues for determination. Recently, EAG initiated research to determine the best way to measure such constants in vitro as well as mathematical models for incorporation into global PB-PK models. Results suggest that several models could be used for incorporating in vitro derived values. Predictive ability is a function of the extraction ratio of the compound. Depending on the approximate value of the extraction ratio, differences between the models apparently may or may not be significant. An expert system will be drafted that will draw on a developed knowledge base of rules and extraction ratio values stored in the database to reach conclusions regarding the best mathematical in vitro to in vivo extrapolation model.

Another option is to build an expert system that helps the user decide which type of pharmacokinetic model is needed for a reconstructive dose/exposure assessment, given certain site-specific data.

From this work and the PB-PK model summaries, other expert systems would be developed to help choose other components of model formulation. Ultimately, an extensive expert system would be constructed that would encompass the several phases of model building.

Identified Results: *For the first two years of the project only:*

Short-term - Summaries of types of PB-PK models and the conditions for which they are appropriate. Prototype expert system to select the best mathematical model for incorporating in vitro rate constants into in vivo models. Prototype expert system to decide the type of pharmacokinetic model that is most feasible for a reconstructive dose/exposure assessment.

Long-term - Guidance on model formulation criteria and expert system to aid in model formulation and simplification.

Usefulness of Results: This project will help establish a consensus on the best way to formulate and validate PB-PK models. In addition, the expert system approach will encourage all users to take full advantage of existing data. Such an approach will ensure that, because of the inherent logic in expert systems, the validity of assumptions used in model formulation can be accurately evaluated. Expert systems can simultaneously accommodate qualitative and quantitative information in the decision-making process.

Project Length and Cost: 3 yr FY89: $50K

Project Officer: P. White (OHEA-EAG) (202) 382-2589

Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.3 Theoretical Models

Status: Ongoing

Title: Computational Molecular Models for the Deposition, Disposition, Transformation, and Activity of Specific Chemical Classes

Description:

The action of a xenobiotic chemical in a biological system results from its interaction with the molecules in the biological system. Its deposition, transformation, disposition, and activity at its molecular site of action depend on its specific interaction with endogenous molecules and nonspecific chemical processes. All of these microscopic actions determine the macroscopic activity of the xenobiotic. The interactions and nonspecific processes that determine these actions depend on the same physicochemical properties that determine chemical reactions.

In this project, computational models will be developed based on a description of the underlying molecular processes that determine the pharmacokinetics and pharmacodynamics of specific chemical classes. A class is defined as chemicals that appear to act by the same mechanism, not just by structural similarity. Members of classes for which relevant data exist, as well as for which we have knowledge of the underlying biomolecular processes, will be used to initiate the modeling. Emphasis will be placed on any available information about the molecular processes that determines differential activity within the class. These models will then be used to predict the pharmacokinetics and activity for untested chemicals within the class. The transformation of polyaromatic hydrocarbons to their active intermediates and the stability of these intermediates will be the relevant molecular process modeled to initiate this effort. Assessment of the potential for health effects from individual chemicals within a class is important for Superfund and other Program Offices.

Quantum mechanics, along with methods to compute physical properties, will be used to model the relevant molecular characteristics. Many of the relevant methods already exist or are being developed in other tasks.

Another relevant class of chemicals for study by these methods is the short-chain acids and chemicals that are transformed to short-chain acids (e.g., glycol ethers, phthalates, alcohols). For these chemicals, the important processes to be modeled are the transformation deposition and activity. A model for the substituted phenols will be developed to compare with the results from Project 3.1.1(1). As the project progresses, other chemical classes may be added.

Identified Results:

1. Methods to predict the transformation, stability, and covalent bonding to biopolymers of the active intermediates of PAHs and similar chemicals. Intermediate journal articles on these methods and applications to specific chemicals in the class.

2. Models for the transport and activity of short-chain acids and molecules that are transformed into short-chain acids. These models could be used to perform assessments of specific chemicals within a class and as a tool for understanding the health effects of class members.

3. Models for the transport or activity of chemical classes as yet unspecified, particularly classes of chemicals for which the health effects are receptor-mediated (e.g., dioxins, some pesticides).

Usefulness of Results: In many cases, risk assessments must be performed without all the requisite data. In some instances, even data on the physicochemical properties are unknown. Causal computational models that predict from molecular structure the distribution, transformation, and molecular-level activity of the members of specific chemical classes will provide rational input to these assessments. These models will also aid in the design of a bioassay strategy for the chemical class that rationally treats assessment needs and should operate in a feedback mode with the development of bioassay data (for instance, the data on substituted phenols).

The knowledge gained about the underlying mechanism of action will aid in extrapolation from bioassay data to situations removed in dose regime, species, etc., from the experimental circumstance. PAHs, the initial class of chemicals for study, are important for Superfund, Hazardous Waste, and other Program Offices. The class of chemicals that includes short-chain acids and the chemicals transformed to short-chain acids is of importance to Toxics, Superfund, and perhaps other Offices. At least two, possibly related endpoints in this class are of interest for assessment. If other classes of chemicals are chosen for modeling, both the chemical class and endpoint will be chosen because of their importance to risk assessment needs.

Project Length and Cost: 3 yr FY89: $150K

Project Officer: J. Rabinowitz (OHR/HERL-RTP) (919) 541-5714

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.3 Theoretical Models

Status: Ongoing

Title: Embryonic Dosimetry and Pharmacokinetic Studies in Developmental Toxicity

Description: Within the Perinatal Toxicology Branch, a major research project is underway to evaluate structure-activity relationships for the induction of developmental toxicity in several chemical classes (para-substituted phenols and branch-chained carboxylic acids) using both in vivo (Chernoff/Kavlock Assay) and in vitro (whole embryo culture) bioassays. Biological activities of 27 phenols in vivo and a subset of 12 in whole embryo culture have been completed and submitted as an Administrator's Item in August 1988. That work is attempting to link specific molecular parameters (e.g., lipophilicity, electronic effects of substituents, molar refractivity) with the potential for causing adverse effects in the conceptus.

Building on this large database, this project will establish basic pharmacokinetic data on the processing of the congeners in the two systems, obtaining information on metabolism, maternal plasma kinetics, whole body clearance, and deposition of the congeners at the target site within the embryo. The structure-activity studies, with their emphasis on cross-assay comparisons of effects, suffer from the inability to determine exactly what concentrations are being delivered to the embryo. Potency measurements are now based on administered dose, and improvements in quantifying the structure-activity relationships should be expected if the potencies can be expressed on delivered dose. This project will

provide the resources to establish the link between administered dose, delivered dose, and biological effect for a well-defined set of chemical congeners.

Identified Results: Physiologically based models of a series of chemical congeners in the maternal/fetal unit. These models should provide direct evidence of how molecular properties affect placental passage and embryonic development within the chemical class under study. Using the in vitro system, this effort will determine the role of metabolism in the induction of adverse effects, and these data will then be compared to in vivo observations. This work will produce the first side-by-side analysis of the chemical dosimetry and reactivity in in vivo and in vitro assay systems. The first chemical class for study is also the subject of structure-penetration studies for the dermal route of exposure; therefore, the data derived from the oral studies in this proposal will provide information on route-route differences in pharmacokinetics.

Usefulness of Results: To improve the methods by which teratogenic risk is currently extrapolated, attention must be placed on determining those factors that contribute significantly to the interspecies differences in the teratogenic responses that are observed following chemical exposure. These factors must be either pharmacodynamically and/or pharmacokinetically based; and determining their relative importance, even for one or two chemicals, is an enormous undertaking. This project will provide a unique pharmacokinetic database that relates the influence of physical-chemical properties in deposition in the maternal/embryonic unit at the time of critical susceptibility and how that aspect affects the expression of developmental toxicology. Combining the in vivo and in vitro data will provide important direct information on the interpretability of the embryo culture system. The project can be expanded in the future to provide comparable data for the same congeners in other whole animal and in vitro developmental toxicity assays.

Project Length and Cost: 3 yr FY89: $200K

Project Officer: R. Kavlock (OHR/HERL-RTP) (919) 541-2326

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Topic: Physiologically Based Pharmacokinetic Models

Issue: 3.3 Theoretical Models

Status: Ongoing

Title: Pharmacokinetic and Biological-Time Based Models for Teratogenesis

Description: Pharmacokinetic models for fetal concentration, based on published and unpublished data, have been developed for simulation of maternal-blood concentrations and fetal concentration of hydroxyurea in rats and primates. When the dose is expressed in terms of biological time (units of percent of the period of organogenesis) instead of real time, the estimated potency (response) seems to be consistent with the experimental response data. Several organic acids (e.g., trichloroacetic acid) have been identified as the proximate teratogen. Both pharmacokinetic and teratogenic data from both primates and rodents regarding maternal blood concentration and fetal tissue and fluid concentrations (sometimes in terms of both parent compounds and reactive metabolites) are available.

The apportionment between the fetal fluids and the fetal tissue is probably the result of the pk_a of the acid. Changes in the cell turnover are influenced by the cellular pH.

Unpublished information will be gathered. Data on fetal body weight and amounts of fetal fluids during the period of organogenesis as well as actual combined fetal response (instead of divided response) will be gathered. Models will be developed based on maternal blood concentration, fetal fluid concentration, and pk_a-influenced fetal concentration. Simulations will be compared to experimental results concerning fetal concentration and response when the dose is expressed in terms of biological time. The use of cell-turnover-based models will be explored as a basis for adjusting the sensitivity of the fetus with respect to teratogenic responses and, perhaps, in utero exposure to carcinogens.

Identified Results: Collation of important pharmacokinetic and physiologic data such as fetal body weight and sizes of fetal fluids during the period of organogenesis and actual combined fetal response instead of divided response: fetal death, specific incidence of anomalies. PB-PK models describing disposition in the fetus and pharmacodynamic models using cell-turnover rates as an endpoint for sensitivity determination.

Usefulness of Results: No pharmacokinetic and pharmacodynamic models relating maternal exposure to fetal outcome exist. This project will provide a basis for developing a methodology for rationally assessing the possible damage to the embryo/fetus. This proposal deals with higher-resolution endpoints than simple placental crossing to assess fetal/embryonic sensitivity to teratogens and intrauterine carcinogens.

Project Length and Cost: 2 yr FY89: $70K

Project Officer: R. Beliles (OHRA-HHAG) (202) 382-5898

APPENDIX D
TOPIC 4: BIOLOGICALLY BASED DOSE-RESPONSE MODELS PROGRAM OVERVIEWS AND PROJECT DESCRIPTIONS

D.1 NEUROTOXICOLOGY

Increasing emphasis and efforts have been placed in the past few years on identifying and characterizing the hazards of neurotoxic chemicals. These developments have come about largely as a consequence of a growing recognition of the number of chemicals that have been either shown or suspected to produce behavioral and/or neurological impairments in humans. Among the evidence gathered are data from laboratory studies indicating that many chemicals cause a variety of neuronal impairments, ranging from subtle functional changes to frank structural alterations. These trends have been well documented in the scientific literature and have led a number of expert scientific associations and committees to recommend an increased commitment to identifying, characterizing, and regulating neurotoxic chemicals.

Recently, EPA has also focused an increasing amount of attention on neurotoxicity. In 1985, for example, the Office of Toxic Substances (OTS) published a broad set of test guidelines for evaluating neurotoxic compounds. Many of these guidelines are now being applied, either through test rules or consent agreements, to the evaluation of compounds under section 4 of the Toxic Substances Control Act (TSCA). Some of these methods are also being applied to the evaluation of compounds under section 5 of TSCA, for example through the Data Gaps project of OTS. Additional guidelines have either been promulgated (e.g., developmental neurotoxicity) or are under development (e.g., electrophysiology). Intra-Agency committees that include Neurotoxicology Division (NTD) scientists are being formed to review existing guidelines and make recommendations concerning the need for additional guidelines. The heightened Agency awareness of neurotoxicity as a health effect of regulatory concern has highlighted the need for neurotoxicity risk assessment guidelines. An intra-Agency workshop that includes NTD scientists has also recently been formed to develop guidelines for making risk assessments on the basis of neurotoxicity data.

D.1.1 Neurotoxicology Division Research

The Neurotoxicology Division has developed and maintained a program of research to provide scientific support to the activities of the Program Offices. Broadly speaking, NTD has ongoing programs to: 1) develop and validate methods for identifying and characterizing neurotoxic hazards; 2) provide data on the neurotoxicity of key chemicals or chemical classes of concern to the Program Offices; and 3) produce an empirical basis for interpreting the significance of laboratory results and extrapolating them to humans. In the course of these activities, NTD has recognized that the risk assessment process can be improved by a research program targeted to address certain recurrent generic issues of concern. To develop such a program, NTD has both undertaken new research directions and refocused a portion of its ongoing research. A description of recently initiated and proposed efforts follows.

D.1.1.1 Current RIHRA Projects

1. Considerable effort is being focused on the issue of inter/intra-species extrapolation (Issue 04). Particular emphasis is being placed on empirically determining the degree of homology that exists between human sensory and cognitive processes and their animal counterparts (Issue 0401). These studies involve direct laboratory comparisons of human and animal data using neurophysiological and behavioral test methods. A successful outcome of these studies will greatly enhance our confidence that results obtained from laboratory animals are predictive of effects seen in humans under similar exposure conditions.

2. A portion of NTD's research also focuses on a direct comparison of the susceptibility of rats and hens to organophosphate-induced delayed neurotoxicity in order to determine whether rats can be substituted for the traditional "hen test." Successful completion of this project will provide an empirical basis for specifying rats as an alternative and biologically more significant species for identifying neuropathic organophosphates.

3. Research is also underway to determine intra-species differences in sensitivity to neurotoxic compounds (Issue 0403). This research is specifically designed to compare basic neurobehavioral processes in F344 and Long-Evans rats using behavioral and biochemical test methods. This research will be supplemented by electrophysiological tests of nervous system function. Because industry and contract laboratories frequently use F344 rats in toxicity testing but NTD research has focused almost exclusively on Long-Evans rats, data gathered in this project will be significant for species comparisons and cross-species extrapolation. For example, the F344s are albino rats known to have neurological anomalies, and Program Offices need to know whether the neurotoxicity data collected with these rats are indicative of effects seen in other strains of rat such as Long-Evans.

4. Research is also underway in NTD to determine the extent to which the manifestations and mechanisms of neurotoxicity depend on the particular exposure conditions (Issue 0403). Specifically, this project is designed to determine the relationship between level and duration of exposure in producing neurotoxicity using behavioral, biochemical, and morphological test methods. This research will provide an empirical basis for predicting neurotoxicity under one set of exposure conditions on the basis of data available for another set of exposure conditions. This research will also indicate whether short-term high-level exposure studies may be substituted for the more costly long-term low-level studies currently required by OTS and the Office of Pesticide Programs (OPP).

D.1.1.2 Proposed Projects

The proposed projects were selected to both augment and complement current RIHRA efforts.

1. The project on quantitative risk estimation directly supports multiple RIHRA issues. This project is specifically designed to evaluate and compare new methods for quantitatively estimating risk with the traditional approach of determining No Observed Effect Levels (NOELs) and then applying uncertainty factors. Considerable interest has been displayed in the scientific community and within the Agency in this type of approach. For example, in Cincinnati Dourson and colleagues are currently evaluating alternative methods for quantitative risk estimation, but these do not include applications to neurotoxicology. The results of this project will therefore not only complement ongoing efforts within the Agency, but will also provide a basis for further applications and evaluations using additional methods for assessing neurobehavioral integrity.

2. The project on homologous models of sensorimotor function is specifically designed to compare the results of reflex testing in humans and in animals. The particular sensorimotor reflex chosen for this study has already been shown to occur in a wide range of species and to be sensitive to a variety of toxicants in laboratory animals. Owing to its noninvasive nature, the technique offers great promise for rapid and accurate neurotoxic hazard identification and characterization in human populations. This project will complement current efforts to evaluate homologies in sensory processes using electrophysiological techniques, and in cognitive processes using behavioral techniques.

3. The project on the role of age as a determinant of the sensitivity and severity of effects of cholinesterase-inhibiting pesticides was designed to specifically address a research need recently identified by the Technical Panel on Cholinesterase Inhibition to the Risk Assessment Council (12 September 1988 memo from P. Preuss to V. Newill). This project will provide needed information on the importance of including an additional uncertainty factor, based on age of exposure, in assessing the neurotoxic risks posed by this class of pesticide.

4. Finally, the project on historical data is designed to assemble existing pharmaceutical data on the neurobehavioral effects of centrally acting chemicals in multiple species, including humans wherever possible. This project will provide direct evidence of the adequacy of traditional uncertainty factors to compensate for variations in susceptibility both within and between species.

In summary, the proposed projects represent a blend of efforts to empirically determine the relationship of neurotoxic effects seen in different species, including humans, and to both improve upon existing methods and identify new and promising methods for risk assessment. The results of these projects will therefore support and complement current efforts within the Program Offices to assess the risks associated with neurotoxic chemicals.

Topic: Biologically Based Dose-Response Models

Issue: 4.1.1 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: Homologous Models for Evaluating Sensory Function

Description: This project is designed to establish the relationship between measures of human sensory function and measures taken from laboratory animals. This problem may be addressed by parallel studies in laboratory animals and humans that directly compare measured endpoints. Sensory-evoked potentials are electrophysiological recordings taken from the sensory areas of the brain following a sensory stimulus that are thought to be good measures of sensory function. They can be recorded from any species, including humans and rats. The problem to be investigated is how closely the rat and human potentials are related, and to what extent changes in one species predict those in the other. This approach involves parallel studies in both species examining: 1) the response to manipulation of stimulus parameters, 2) the response to certain drugs, and 3) the relationship to other measures of sensory function. If sensory-evoked potentials recorded from man and rats show similar changes in response to stimulus and drug treatments, then there will be better grounds for concluding that the responses measured from the two species reflect similar functional mechanisms, and that changes reported in the sensory-evoked potentials of animal species are predictive of changes in human sensory function.

Identified Results:

1. Internal progress reports
2. Final report describing the nature of the project, the results, and recommendations for using these types of sensory assessments in determining the risks for humans exposed to neurotoxic chemicals. These reports will be supplemented by peer-reviewed publications in the open literature.

Usefulness of Results: A lack of homologous models for neurotoxic endpoints has been identified as one of the major sources of uncertainty in the risk assessment process (RIHRA). The Program Offices of EPA, including OPTS, must decide the likely human consequences of exposure to compounds based on animal data, even though the extent to which the measured endpoints in the animal species coincide with adverse consequences in humans is often unknown. This problem may be addressed by parallel studies in laboratory animals and humans that directly compare measured endpoints. If measures of sensory function recorded from man and rats shown similar changes in response to a variety of treatments, then EPA will have better grounds for concluding that the responses measured from the two species reflect similar functional mechanisms. Successful completion of this project should improve the ability to perform cross-species extrapolation of sensory neurotoxic effects. This information will help Program Offices, including OPTS, determine the extent to which changes reported in animal species are predictive of changes in sensory function of humans.

Project Length and Cost: 3 yr FY89: $122K

Project Officer: K. Hudnell (OHR/HERL-RTP) (919) 541-7538

Topic: Biologically Based Dose-Response Models

Issue: 4.1.1 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: Homologous Models for Assessing Learning and Memory

Description: This project is designed to improve animal-to-human extrapolation in the study of chemical-induced learning and memory disorders, in order to reduce uncertainty in evaluating potential risk posed by chemicals for producing these disorders in adult and developing organisms. To accomplish this objective, we will employ two basic learning and memory paradigms that have recently been used in comparative studies of cognition involving animals and humans. Research with human infants asks whether a range of memory phenomena that can be demonstrated with these tests emerge during ontogeny in a manner which would be predicted from maturation of their neural substrates and/or maturational profiles of behavior that occur in non-human animals. Additional work with animals will attempt to alter memory development by disrupting neural maturation with lesions, CNS teratogens, neurotoxicants, and environmental chemicals with known or suspected neurotoxicity. In human infants, we are trying to identify special "high risk" subject populations, neurological cases, or victims of accidental chemical exposure for study. In human adults, we will examine task performance in healthy volunteers administered various neurally active drugs, in neurological populations, and in populations subject to occupational chemical exposure. Parallel studies will be conducted in animals that will model the chemical treatment or neurological insult.

Identified Results: The products will include internal progress reports and a final report describing the nature of the project, the results and recommendations for using these types of cognitive assessments in determining the risks for humans exposed to neurotoxic chemicals. These reports will be supplemented by peer-reviewed publications in the open literature.

Usefulness of Results: Cognitive effects are often reported in humans following exposure to neurotoxic environmental pollutants. To predict human cognitive impairments, risk assessors must usually evaluate data obtained from laboratory animals. The tests applied to laboratory animals often appear similar to tests which can be applied to humans, and many superficially parallel methods for evaluating these kinds of effects are currently in use. However, it is not clear to what extent changes recorded in animals can be extrapolated to humans, and risk assessors will therefore have difficulty using animal data. These studies are designed to determine the extent to which certain forms of learning and memory share similar properties in animals and humans, and the extent to which they may share a common neural substrate. Understanding these relationships will reduce uncertainty in inferring human neurotoxic risk based on animal data.

Project Length and Cost: 3 yr FY89: $85K

Project Officer: M.E. Stanton (OHR/HERL-RTP) (919) 541-7783

Topic: Biologically Based Dose-Response Models

Issue: 4.1.1 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: Learning and Memory in Human Neonates

Description: This project is designed to improve animal-to-human extrapolation in the study of chemical-induced learning and memory disorders, in order to reduce uncertainty in evaluating potential risk posed by chemicals for producing these disorders in adult and developing organisms. Specifically, this project represents the human infant component of OHR Task #1375. In order to accomplish this objective, we will employ two basic learning and memory paradigms that have been used in comparative studies of memory involving animals and humans. Research with human infants asks whether a range of memory phenomena that can be demonstrated with these tests emerge during ontogeny in a manner that would be predicted from maturation of their neural substrates and/or maturational profiles of behavior that occur in non-human animals. In addition to understanding the basic behavioral processes, this project will also improve our understanding of the degree to which cognitive deficits in humans can be modeled using laboratory animals. We will try to identify special "high risk" human infant populations, neurological cases, or victims of accidental chemical exposure for study.

Identified Results: The products will include progress reports and a final report describing the nature of the project, the results and recommendations for using these types of cognitive assessments in determining the risks for human infants exposed to neurotoxic chemicals. These reports will be supplemented by peer-reviewed publications in the open literature.

Usefulness of Results: Cognitive effects are often reported in humans following exposure to neurotoxic environmental pollutants. To predict human cognitive impairments, risk assessors must usually evaluate data obtained from laboratory animals. The tests applied to laboratory animals often appear similar to tests which can be applied to humans, and many superficially parallel methods for evaluating these kinds of effects are currently in use. However, it is not clear to what extent changes recorded in animals can be extrapolated to humans, and risk assessors will therefore have difficulty using animal data. This project is part of a larger series of studies designed to determine the extent to which certain forms of learning and memory share similar properties in animals and humans, and the extent to which they may share a common neural substrate. Understanding these relationships will reduce uncertainty in inferring human neurotoxic risk based on animal data.

Project Length and Cost: 2 yr FY89: $110K

Project Officer: M.E. Stanton (OHR/HERL-RTP) (919) 541-7783

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.1 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: Cross-Species Validation of a Risk Assessment Method for the Characterization and Prediction of Peripheral Neuropathy

Description: This project is designed to determine the relationship between a neurotoxic syndrome seen in two different species of test animal. Specifically, this project will compare the neurochemical and neuropathological effects of organophosphate esters in hens and rats to determine the extent to which inhibition of neurotoxic esterase in both species is predictive of later pathology. This research is necessary to validate the use of rats as an alternative test species to screen for neuropathic organophosphate esters. Rats and hens will be exposed acutely to various organophosphorus-containing compounds as well as additional selected toxicants. Levels of inhibition of neurotoxic esterase (NTE) will be determined in the brain and spinal cord of each species shortly after dosing (24-48 hr). Additional animals will be sacrificed approximately two weeks later for neuropathological assessment of the peripheral nervous system and spinal cord. The extent of pathology will be correlated with the degree of NTE inhibition and compared across species. Compounds selected for this validation effort include a matrix of those that either do or do not produce inhibition of NTE and those that either do or do not produce later neuropathology.

Identified Results: The products will include internal progress reports and a final report describing the nature of the project, the results and recommendations for substituting rats for hens as the required species for identifying neuropathic organophosphorus-containing compounds. These reports will be supplemented by peer-reviewed publications in the open literature.

Usefulness of Results: Many industrial and pesticidal formulations contain organophosphates, and many organophosphorus compounds produce delayed neuropathy. EPA routinely requires the hen test for evaluating the potential of a compound to produce this type of neuropathy. However, the hen is not used for the other forms of testing required by the Agency. Historically, the rat has been considered refractory to this type of neuropathy. However, recent evidence from NTD suggests that rats are indeed susceptible. This research will be directed to determine the extent to which organophosphate-induced delayed neuropathy is similar across species. More specifically, the research will determine the extent to which rat data can be used as a more efficient and biologically based substitute to predict human risk.

Project Length and Cost: 3 yr FY89: $225K

Project Officer: S. Padilla (OHR/HERL-RTP) (919) 541-3956

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.3 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: Intraspecies Differences in Susceptibility to Chemical-Induced Neurotoxicity

Description: This project will help determine whether it is necessary to specify a particular type of rat in assessing the neurotoxicity of chemicals. To accomplish this objective, neurobehavioral function will be compared in F344 and in Long-Evans rats. F344 is the strain that is widely used in routine toxicity testing, whereas Long-Evans rats are one of the most widely used stocks in neurotoxicology research. The types of neurobehavioral function assessed will include temperature regulation and metabolism, learning, and performance. Concurrent measurements of neurotypic and gliotypic proteins will provide biochemical markers of nervous system integrity. Parametric studies will assess whether the neurobehavioral functions, as well as distribution and levels of neurotypic and gliotypic proteins, are similar in both strains of rat. Chemical exposures using prototype neurotoxicants will next be arranged and the responses of the two species will be compared. Analytical chemistry will be employed to determine whether brain levels of neurotoxicants producing effects are comparable in the two species.

Identified Results: This project will result in internal progress reports and a final report describing the test conditions, results, and recommendations for requiring a particular species of rat for neurotoxicity testing purposes. These reports will be supplemented by peer-reviewed journal articles in the open literature.

Usefulness of Results: Although albino strains such as the F344 are widely used in routine toxicity assessments, many albinos have neurological abnormalities suggesting they may be inappropriate for neurotoxicity testing. By determining whether qualitative and/or quantitative differences exist in neurobehavioral function between F344s and Long-Evans rats, and whether the two species are affected similarly by neurotoxicant exposures, this project will give risk assessors an empirical basis for stipulating the particular type of rat to be used in neurotoxicity testing.

Project Length and Cost: 3 yr FY89: $81K

Project Officer: R. MacPhail (OHR/HERL-RTP) (919) 541-7833

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Topic: Biologically Based Dose-Response Models

Issue: Multiple Issues

Status: New

Title: Quantitative Risk Assessment in Neurotoxicology

Description: This project is designed to determine the relative value of traditional and novel approaches to quantitative risk assessment based on neurobehavioral data. Laboratory animals will be exposed to neurotoxic environmental pollutants, and dose-response functions will be established using behavioral measures of neurotoxicity. The experimental design will vary the number of animals used per exposure group and the amount of variability in behavioral performance obtained within and between animals. The resulting dose-response curves will be analyzed mathematically using curve-fitting techniques in order to calculate ADIs. The resulting ADIs will be compared to those determined using the traditional approach of adjusting NOELs using uncertainty factors.

Identified Results: This project will result in internal progress reports and a final report describing the test conditions, models, results, conclusions, and recommendations for using these two approaches for quantitative risk assessment using neurobehavioral endpoints. The conclusions and recommendations will be supported by peer-reviewed journal articles in the open literature.

Usefulness of Results: The traditional approach to risk assessment involves determining NOELs, then adjusting exposure levels downward with uncertainty factors in order to arrive at an ADI. For example, the NOEL is routinely divided by 10 in order to compensate for individual differences in sensitivity. An alternative approach to risk assessment has recently been introduced (e.g., Crump, 1984; Dews, 1986) in which the ADI is calculated by fitting mathematical models to dose-response data, and then estimating the dose corresponding to some specified small amount of additional risk. This project will directly compare the utility of these two different approaches for making risk assessments in neurotoxicology. Program Offices (e.g., OPTS) are beginning to require submission of data on the neurotoxic potential of chemicals. The products of this project will therefore greatly improve the Agency's ability to make risk assessments in order to efficiently and effectively regulate levels of exposure to neurotoxicants.

Project Length and Cost: 3 yr FY89: $120K

Project Officer: R. MacPhail (OHR/HERL-RTP) (919) 541-7833

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.3 Inter/Intraspecies Extrapolation: Interspecies Sensitivity

Status: Ongoing

Title: Age as an Uncertainty Factor in Assessing Sensitivity and Severity of Effects Produced by Cholinesterase-Inhibiting Compounds

Project Status: New

Description: The objectives of this project are twofold. First, the project will determine the extent to which the severity of effects produced by cholinesterase-inhibiting compounds is dependent upon the age of exposure. Second, the project will determine whether there are any principles (e.g., metabolism) governing the extent to which different age subjects are variously sensitive to the cholinesterase-inhibiting properties of cholinesterase inhibitors. Data will be collected in rats. To address the severity question, different groups of rats will be exposed to a variety of cholinesterase-inhibiting compounds during different developmental stages, from prenatal to late adulthood. The relationship between level of cholinesterase inhibition and neurobehavioral and neurochemical effects will be compared across age groups. To address the sensitivity question, the relationship between exposure and cholinesterase inhibition will be explored during different developmental stages. Neurobehavioral endpoints to be measured will be determined between NTD staff and the awardee.

Identified Results: Two products are envisioned, both reports. One report would provide an assessment of the extent to which age affects sensitivity to cholinesterase-inhibiting compounds. The second report would provide an assessment of the extent to which age affects the severity of effect produced by equivalent levels of cholinesterase inhibition. These reports will be supplemented by publication of the results in peer-reviewed journals.

Usefulness of Results: Several Program Offices (e.g., ODW, OPTS, OSWER) perform risk assessments on substances whose principal effect is inhibition of the cholinesterase enzymes. For several years, the Risk Assessment Forum has had an active Technical Panel whose mission was to develop a uniform Agency policy for applying uncertainty factors when performing risk assessments on cholinesterase-inhibiting compounds. At a recent colloquium convened by the Risk Assessment Forum to discuss the Technical Panel's report, age-dependent severity and age-dependent sensitivity were highlighted as issues that have not been addressed adequately. Insufficient data are currently available to determine whether any principles can be applied to cholinesterase-inhibiting compounds to address these areas of uncertainty.

Project Length and Cost: 3 yr FY89: $160K

Project Officer: K. Jensen (OHR/HERL-RTP) (919) 541-1560

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.3 Exposure Scenarios: Interaction of Exposure Parameters on Outcome

Status: Ongoing

Title: Exposure Scenarios and Neurotoxic Outcome

Description: This project is designed to determine how variations in exposure may affect the neurotoxicity of chemicals. To accomplish this objective, we will determine how level and duration of exposure to neurotoxic compounds jointly affect nervous system integrity. Laboratory animals (rats) will be exposed to neuropathic chemicals and nervous system integrity will be evaluated using morphological, biochemical, and functional (neurobehavioral) tests. Systematic variation of both the level and duration of neurotoxicant exposure will be used to determine whether neurotoxic outcome varies with exposure scenario. Analytical chemistry will also be included to determine whether exposure-related changes in neurotoxicity are due to differences in target-site sensitivity or a simple accumulation of the toxicant at the target site.

Identified Results: This project will result in internal progress reports and a final report describing the nature of the project, results, and recommendations regarding the extent to which neurotoxic effects under one set of exposure conditions can be extrapolated to other exposure conditions. These reports will be supplemented by peer-reviewed journal articles published in the open literature.

Usefulness of Results: Risk assessment often involves predicting neurotoxic effects following long-term, low-level exposure on the basis of data from short-term, high-level exposures. Uncertainties in predicting neurotoxic hazards could be greatly reduced by empirically determining the

relationship between level and duration of exposure, and whether the mechanism(s) underlying neurotoxicity change as a function of exposure scenario. An additional potential benefit of this project is the possibility of being able to require short-term, high-level exposure testing in lieu of more costly longer term, low-level exposure testing.

Project Length and Cost: 3 yr FY89: $106K

Project Officer: K. Crofton (OHR/HERL-RTP) (919) 541-2672

D.2 DEVELOPMENTAL/REPRODUCTIVE TOXICOLOGY

This section provides the background and rationale for the areas of focus for the reproductive, developmental, and germ cell mutagenesis research under RIHRA. It represents a synthesis of HERL and OHEA directions and reflects activities in the existing FY89 OHR RIHRA and OHEA/RURA base programs as well as newly funded projects.

Throughout the existence of the EPA, Program administrators have been faced with making decisions on the potential of environmental pollutants to adversely affect the reproductive processes of humans. The Agency developed animal testing guidelines for reproductive and developmental toxicity in the late 1970s and, six years ago, published them in final form (Pesticides Assessment Guideline, Subdivision F. Hazard Evaluation: Human and Domestic Animals, EPA-540/9-82-025). Minor modifications to the protocols were made later by the Office of Toxic Substances. In addition, the Office of Toxic Substances has recently indicated a commitment to require testing and regulation on the basis of heritable genetic risk (Fed. Reg. 53L247:51, 847-851, 856, 1988).

The risk assessment process for developmental toxicants was standardized in 1984 with publication in the Federal Register of the "Proposed Guidelines for Health Assessment of Suspect Developmental Toxicants." These guidelines were issued in final form in 1986 and revised and published for public comment in Spring, 1989. Final publication is planned for 1990. Proposed guidelines for Male Reproductive Risk Assessment and for Female Reproductive Risk Assessment were published in the Federal Register in June, 1988. Publication of those guidelines in final form is also anticipated in 1990. Guidelines for germ cell mutagenicity risk assessment were published in final form in Fall, 1986. The current status of reproductive, developmental, and mutagenicity risk assessment is described in these sets of guidelines.

These documents also delineate fundamental research areas for which the directing of resources would most likely improve our ability to use animal testing data in risk assessment. In particular, the developmental and reproductive risk assessment guidelines describe several areas of uncertainty inherent in the risk assessment/risk characterization process that could benefit from a well-designed research program. An integrated program would evaluate those processes necessary to maintaining the reproductive capability of each parent, including the production of normal offspring.

In germ cell mutagenesis research, investigations on the male risk have dominated: the proposed program should encompass research on both sexes. Furthermore, the RIHRA program will be coordinated with the National Toxicology Program, which is recognized as a major research program in this field.

The preparation of the developmental, reproductive, and mutagenicity guidelines has benefited from a number of recent workshops, symposia, and position papers (Agency-sponsored and otherwise) that have identified critical research needs. Part of this effort has included commissioning a document that provides a detailed critique of research needs in 16 different areas of developmental toxicology. Not only does this document form a logical basis for expanding the existing EPA research efforts in developmental toxicology, but many of these research areas have high priority in reproductive risk assessment as well.

In discussions between the developmental, reproductive, and genetic toxicologists of OHR and OHEA, several mutually agreeable research needs were identified in accordance with the issues described in Topic 4. The key areas concentrated on homologous mechanisms to strengthen inter-species

extrapolation (Issue 4.1.1) and the development of biologically based dose response models for high-to-low dose extrapolation. Elements of this latter activity relate to Issues 4.2.1 and 4.2.2 and include ascertaining the interrelationship and co-influence of different events expressed over the dose-response range (e.g., the relationship between maternal and developmental toxicity); establishing the predictive validity of low-dose, sensitive measures to higher dose, functional outcomes; and identifying basic mechanisms and determining the commonality of such mechanisms across dose. These activities are viewed as critical to deriving the most valid dose-response function upon which to base BB-DR models.

The priority and level of inquiry into each of these issues was a function of toxicologic, physiologic, and mechanistic knowledge available in the areas of male and female reproduction and developmental toxicity as well as the relationship to base program activities. Thus, extensive activity in the delineation of mechanisms and dose-response modeling is proposed for developmental toxicity—an area that would benefit from a more substantial database. On the other hand, the reproductive toxicology efforts concentrate primarily on obtaining fundamental information on species comparability and endpoint interrelationships. BB-DR modeling is seen as a subsequent undertaking in reproductive toxicology.

The assessment of female reproductive risk is the least developed of these areas and poses the greatest uncertainties. Thus, emphasis in this area is directed toward defining the mechanistic homology between conventional test species and humans for the critical events of ovulation, fertilization, embryo-uterine interactions prior to implantation, and the implantation process (Issue 4.1.1). Work in this area will improve our ability to predict basic reproductive failure in the human female, including very early pregnancy loss. Equally important, this work will examine the dose-response sensitivity and relationship of the various endpoints for these events (Issues 4.2.1 and 4.2.2). These latter topics have high priority in the male reproductive and developmental areas as well.

The research proposed in the male reproductive area also addresses issues of species comparability but has advanced to a more quantitative level, i.e., the actual derivation of interspecies extrapolation factors. Proposed work in rodents on agents known to alter human sperm production (e.g., via endocrine mechanisms) will provide dose-response data that can be compared to human data obtained in extensive work sponsored by OHEA under its RURA program. Such information will be useful in calibrating the inter-species uncertainty factor for this target and provide important basic information for BB-DR modeling for male reproductive toxicants.

Additional inter-species research is directed at delineating the predictive relationship between normal sperm number and fertility. In test species, sperm measures are more sensitive than fertility indices and are endpoints that can be more readily evaluated than reproductive success in humans. However, the relationship between sperm measures and male fertility is unknown. The proposed projects address this issue in rat, rabbit, and man and will provide critical data for interpreting the biologically functional significance associated with sperm alterations. Such information will promote the use of these measures in the regulatory process by reducing the uncertainty as to health significance associated with such data. Again, the clarification of endpoint interrelationships will contribute to subsequent development of BB-DR models.

The developmental toxicology research concentrates primarily in two areas: 1) endpoint interrelationships (e.g., the relationship between maternal and developmental toxicity) and 2) the generation of BB-DR models including research to test basic mechanistic hypotheses. Laboratory efforts related to the former activity will complement and extend a current OHEA/RURA project that is evaluating data from the NTP continuous breeding and developmental toxicology programs. This effort is examining the relationship between parental toxicity and reproductive/developmental consequences. The modeling efforts and associated laboratory activities will address several issues including the incorporation of the threshold concept. These efforts in BB-DR modeling are particularly timely. The sole application of the RfD approach for noncancer endpoints has been criticized within and outside the Agency. However, recent efforts to develop other statistical approaches have only been partially successful since they also fail to incorporate basic biological processes. The work proposed in the area will provide a more biologically defensible basis for estimating human developmental risk.

As described in the "Guidelines for Mutagenicity Risk Assessment," concern is widespread that exposure of humans to environmental mutagens may induce genetic lesions which can be expected to result

in higher frequencies of spontaneous abortion, lower birth weight, congenital anomalies, and genetic disease among offspring. For example, studies over the last two decades have well documented the substantial contribution of chromosomal anomalies (both structural and numerical) to adverse human reproductive and developmental effects. Thus, research focused on the delineation of mutagenic and other genetic mechanisms relevant to adverse reproductive outcomes is viewed as an important component in reproductive and developmental toxicity research.

Topic: Biologically Based Dose-Response Models

Issue: 4.2.1 Exposure Scenarios: Mechanisms Across Dose

Status: New OHEA/RURA project to be co-funded with HERL

Title: Development of Biologically Based Dose-Response Models for Developmental Toxicity

Description: Past efforts have involved the application of statistical models to data from standard developmental toxicity studies with some attempts to take biological considerations (e.g., litter effects) into account. However, little has been done to evaluate the data available on the underlying biological processes and the toxic effects of particular chemicals and to integrate this into an approach to biologically based dose-response modeling. This project will make use of model developmental toxicants for which some mechanistic/site of actions data are available; incorporate pertinent information on such processes as cell replication, migration, repair, cell death, etc.; and integrate this information with available pharmacokinetic data to derive mathematical models that best incorporate these factors.

This project will involve the solicitation of proposals with new or unique approaches to dose-response modeling in this area. This effort will require the cooperation of basic scientists and risk assessment experts, including experts in biologically based dose-response modelling.

Identified Results:

1. Identification of appropriate model compounds for use in this effort
2. Identification of the basic developmental biology principles that are pertinent to this approach
3. Development of experimental models using the data from 1 and 2 above
4. Identification of data gaps that require additional laboratory efforts

Usefulness of Results: The Agency's guidelines for developmental toxicity risk assessment have called for the development of new approaches to the extrapolation of dose-response data, and many scientists and regulators have criticized the use of the RfD for noncancer endpoints. Although several efforts to develop statistical approaches to this problem have been underway in OHEA and OHR, they only partially deal with the biological processes underlying developmental toxicity. This project could pave the way toward identifying the data available for use in biologically based models and delineating research gaps to be investigated further. Development of appropriate biologically based dose-response models would provide significantly greater confidence in the estimation of human risk.

Project Length and Cost: 3 yr FY89: $160K (50% to be co-funded by HERL)

Project Officers: C. Kimmel (RDTB/OHEA) (202) 382-7331
R. Kavlock (DTD/HERL) (919) 541-2321

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.2 Exposure Scenarios: Sensitivity of Endpoints as a Function of Dose

Status: Ongoing OHEA/RURA project

Title: Quantitative Dose-Response Models for Multiple Endpoints in Reproductive and Developmental Toxicology

Description: The estimation of risk in reproductive and developmental toxicology utilizes primarily a qualitative approach, with the application of uncertainty factors to the NOAEL/LOAEL being the only quantitation currently done. This approach has been criticized by scientists both inside and outside the Agency, who have suggested that a more quantitative approach to estimating human risk is needed.

Several efforts have been undertaken along this line over the past two years, some of which will be continued during the next two years. In particular, a model proposed by Rai and Van Ryzin was applied to data from NTP developmental toxicity studies. This model allows the evaluation of the effect of litter on the final outcome. Refinements in the model were evaluated, and work on the incorporation of the intracluster correlation into the Rai and Van Ryzin model was completed.

More recently, efforts have focused on the relationship of dose-response curves for multiple endpoints in reproductive toxicology. The initial efforts have been to identify the specific parameters to be evaluated and to identify databases available with appropriate data for use in this effort. An IAG with the NTP has been established in order to make use of their databases. The application of the Rai and Van Ryzin model to individual development endpoints has been explored using the NTP database. In addition, through a cooperative agreement with SIMS (Dr. Louise Ryan, Harvard), some analytical approaches for comparing endpoints of developmental toxicity have been explored.

This project will be a continuation of the past efforts in this area, and will focus particularly on the evaluation of endpoint relationships and the development of models for comparing dose-response curves and for low-dose extrapolation.

Identified Results:

1. Identification of the endpoints available for comparison in reproductive and developmental toxicity databases
2. Development of methods for comparison of dose-response curves for multiple endpoints of reproductive and developmental toxicity
3. Development of approaches for combining data from continuous and binomial endpoints of reproductive and developmental toxicity

Usefulness of Results: Quantitative models are needed that appropriately relate dose to response, that relate responses to each other, and that are capable of estimating the risk at any dose level, particularly at the potentially low levels of human exposure. This project will evaluate

the relationship of multiple endpoints of reproductive and developmental toxicity to each other and evaluate the possible influence of some endpoints (e.g., maternal toxicity) on others (e.g., developmental effects). This information will provide an improved understanding of the data from these types of studies which will be invaluable in the risk assessment (both qualitative and quantitative) for reproductive and developmental toxicants.

Project Length and Cost: 3 yr FY89: $40K

Project Officer: G. Kimmel (RDTB/OHEA) (202) 382-5978

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.1 Exposure Scenarios: Mechanisms Across Dose

Status: New RIHRA project

Title: Mutagen-Induced Developmental Toxicity Following Exposure of Mouse Zygotes

Description: Recently, certain mutagens have been shown to induce high incidences of fetal malformation and death when the zygote stage is exposed. This finding raises questions regarding the danger to women who may be exposed to chemicals in the time period immediately following conception. Most toxicologists and clinicians consider the zygote as susceptible to chemically induced toxic effects but relatively impervious to developmental effects. Extensive work has already ruled out maternal toxicity, gene mutations, and chromosomal aberrations as underlying causes of the fetal malformations and death. Nevertheless, a genetic mechanism is still strongly suggested by stage specificity of the effect, as well as by the results of a reciprocal egg-transfer study. It is proposed that this mechanism involves a gene dosage or genetic transposition mechanism.

The implications of this observation, therefore, are extremely important in the assessment of risk for developmental toxicity by showing that the window of susceptibility is not limited to organogenesis and paving the way for mechanistically tying a dose-response relationship with a dose-effect. This project will generate additional information defining the dose-response curve of these mutagen-derived zygote effects and the genetic mechanism(s) involved. Such information is essential for developing risk extrapolation procedures for these mutagen-induced fetal effects.

Identified Results:

1. Data on several different concentrations of a mutagen(s) to allow for the extrapolation of dose-response trends

2. Mechanistic information describing the underlying causes of those trends, which will be useful in developing a model that is biologically reflective

Usefulness of Results: In developmental toxicity risk assessment, a NOAEL/RfD approach to assessing risk is traditionally used. Although a threshold mechanism is usually assumed, the underlying causes of developmental toxicity are largely unclear. Thus, to advance developmental risk assessment in the area of biologically based dose-response models, research is needed to

mechanistically tie dose-response relationships with dose effects. The above proposal is an attempt to accomplish this for mutagen-induced developmental toxicity.

This work is viewed as having a great impact on the approaches that are used to assess developmental toxicity caused by mutagens by providing a framework for generating and testing hypotheses about mechanism. In addition, it will result in an improvement of current risk assessment procedures by extension of the observability of the response to much lower doses.

Project Length and Cost: 2 yr FY89: $80K

Project Officer: V. Dellarco (GTAB/OHEA) (202) 382-7332

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.2/4.2.2 Endpoint Sensitivities and Interspecies Sensitivities

Status: New RIHRA project

Title: Quantitative Extrapolation for Human Male Spermatotoxicity: Relationship Between Production of Normal Sperm and Fertility.

Description: The overall goal is to develop the capability to predict potential effects on human fertility from semen evaluations in humans, from effects on fertility in a test species, or from effects on sperm parameters in a test species.

To predict potential effects on human male fertility, it is necessary to know the quantitative relationships between number of normal sperm produced and fertility in both humans and test species. Additionally, information on the relationships between different measures of sperm production in test species is needed. This project, along with a parallel project with rabbits, will produce those data. Using those data, quantitative extrapolation methods will be developed to replace uncertainty factors in assessment of risk for male spermatotoxicants. Once those methods are established, data from humans or test animals exposed to specific agents or mixtures can be utilized, along with adequate exposure information, to assess potential effects of exposures on fertility of men. This type of approach has been identified as a need in the preparation of the U.S. EPA Guidelines for Assessing Male Reproductive Risk. The approach should be useful in predicting the extent of effects caused by exposure of human males to spermatotoxic agent(s) in or near waste disposal sites, in the workplace or in the environment. Thus, the results of this projects should be of substantial value to the Agency.

In this project, relationships between number of normal sperm produced and time required to achieve conception will be quantified. Human couples desiring to have a child will be recruited. Multiple semen samples will be obtained from each male prior to intercourse at regular intervals around the predicted day of ovulation. Abstinence intervals between ejaculations and timing relative to predicted day of ovulation will be controlled. Multiple endpoints will be examined individually and in combination to assess sperm quality. Urinary estrone and pregnanediol-glucuronides, plus LH and hCG will be measured. Pregnancy will be determined by hCG, missed menses, and by ultrasonic examination.

In the absence of pregnancy, multiple cycles will be studied. Using the data from this study and the parallel project with rabbits, the appropriate conversion factors and equations will be developed to describe the relationships that can then be used to predict effects on fertility.

Specific Aims:

1. Utilize critical analyses of sperm number and quality in men derived from couples attempting conception which will allow determination of the quantitative relationships between number of "normal" sperm and fertility (time required for conception) in humans.

2. Use the data from 1) to predict the effects of reductions in number of "normal" sperm on human fertility (inter-endpoint extrapolation).

3. Combined with the data from the parallel experiments with rabbits, develop extrapolation factors to predict effects on human fertility from effects on "normal" sperm production in rabbits (interspecies extrapolation).

Identified Results:

1. Characterization of the relationships between number of normal spermatozoa produced by human males and measures of fertility

2. Calculation of quantitative extrapolation factors for predicting effects on human fertility from measurement of number of normal sperm produced

3. Combined with results from a parallel project with rabbits, calculation of interspecies extrapolation factors for predicting effects on human fertility from effects on number of normal sperm produced in a test species (rabbit)

Usefulness of Results:

Currently, when risk assessment is conducted involving toxic effect on the male reproductive system, uncertainly factors are applied to a No Observed Adverse Effect Level (NOAEL) to conservatively estimate an exposure level that should be safe for humans. Often, only data from test species are available, and uncertainty factors must be used for interspecies adjustment as well as for protection of more sensitive individuals. Experimentally derived extrapolation factors are preferable to uncertainty factors because they can more accurately reflect the differences found.

This project is designed to contribute to achieving that goal. To do this, the relationships between production of normal sperm and fertility in both humans and test species must first be characterized more rigorously than has been done previously. This project will determine the quantitative relationships between endpoints that reflect sperm number and/or sperm quality and fertility in humans. Efforts elsewhere (independent of this project), will provide similar information with a test species (rabbit). With the interspecies and inter-endpoint relationships available, risk assessments can convert measurements of sperm production to a prediction of fertility status, either when fertility data are not available or when the fertility results are judged to be too insensitive based on other available information. This would have particular application in these situations:

- With suspected human exposures, semen samples may be obtained from the men, but it is unlikely that sufficient information on fertility will be available. If the quantitative relationship between number of normal sperm produced and fertility were known for humans (supplied by data derived from this project), the risk to fertility for men could then be estimated.

- Knowledge of the relationships between number of normal sperm and fertility for both humans and test species can provide the capability to extrapolate between the test species and humans.

- With quantitative extrapolation factors available to convert from results on other endpoints reflecting effects on sperm production, it would be possible to apply inter-endpoint and interspecies extrapolations to predict effects on humans from data on sperm production in a test species. The extrapolation capabilities produced by this project and the parallel project with rabbits can be incorporated into risk assessments that utilize spermatotoxic effects as the critical effect or used to evaluate effects observed in biomonitoring. The approach should be useful for individual chemicals or for mixtures.

In addition to the capabilities described above, this project has important ramifications for in clinical medicine. Those benefits will be obtained from the project as currently designed and will not require any additional expense to EPA. Those ancillary benefits include:

1. Data on the distributions of semen characteristics for a normal population of human males plus fertility information for those same males
2. Data, based on prospective testing, for objectively determining human male fertility parameters
3. Improved ability to diagnose male factor infertility based on laboratory evaluation of semen
4. Improved ability to evaluate the efficacy of certain male contraceptives by evaluation of semen
5. Improved understanding of the timing of events in early pregnancy from use of biochemical and ultrasonic monitoring methods, and ascertain any association with objective laboratory-determined parameters of human sperm
6. Association of the presence of antisperm antibodies with the numbers and normality of sperm found in periovulatory cervical mucus specimens; an indication of the relationship between those properties and fertility may also be obtained

Project Length and Cost: 3 yr FY89: $100K

Project Officer: E. Clegg (RDTB/OHEA) (202) 475-8914

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.1 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: Homologous Models for Very Early Pregnancy (VEP) Loss in Humans

Description:

In mammals, successful pregnancy occurs only when the early developmental events including fertilization and cleavage result in the formation of a normal embryo that is transported to the uterus at the appropriate time and implants normally in a receptive uterus. These early events depend upon the production and time-appropriate release of healthy gametes (sperm and oocytes) and the maintenance of a physiologically sound tubal/uterine environment. Perturbation of any of these events may result in pregnancy termination, even before the pregnancy is detectable.

Recent progress has been made in our ability to detect early pregnancy in humans, most notably by assaying for hCG, the human pregnancy marker, using extremely sensitive immunological tests (Wilcox et al., 1988). Pregnancy surveillance studies using this assay and others, have shown that as many as two-thirds of potential pregnancies fail between the time of ovulation and implantation. The high incidence of VEP loss attests to the vulnerability of early developmental events and the potential for environmental chemicals to act during this period. Indeed, this subject received a great deal of emphasis at a recent NIOSH/NIEHS sponsored symposium on "Assessing Reproductive Hazards in the Workplace" (Cincinnati, OH, June, 1988). Unfortunately, the causes of VEP loss in humans are largely unknown. This is because it is difficult, if not impossible, to access fertilization and early development in women.

In the area of reproductive risk assessment, little or no attention has been given to the characterization of VEP loss in animal models as a means of detecting toxicant-induced VEP loss. This is surprising since both in vivo and in vitro methods for doing so have been available for years. This project will focus on the validation of rodent animal models to evaluate very early developmental events. This approach is justifiable on the grounds that the basic biological/molecular mechanisms underlying early embryonic development and implantation are similar in humans and rodents. Included in the validation process will be the definition of critical periods wherein toxicants may perturb these early developmental events, and determination of mechanisms whereby xenobiotics may cause VEP loss. Obviously, an animal model is needed to produce dose-response data on compounds known or suspected of causing very early pregnancy loss. Initially, compounds with a wide range of potential actions will be examined. These will include compounds that directly perturb cellular processes (e.g. cell division) as well as those that may alter the hormonal milieu during the peri-implantation period.

This approach will also permit discrimination between VEP due to embryo vs. maternal effects. Certainly, the database generated by this approach is critical for the prediction and hopefully characterization of human risks associated with reports of infertility or repeated VEP loss in toxicant-exposed women.

Identified Results:

1. Recommended methods and protocols for assessing toxicant-induced very early pregnancy loss in rodents.

2. Report on critical periods of exposure associated with specific VEP effects.

3. Report on mechanisms underlying VEP loss, common to humans and rodents, at the level of fertilization, preimplantation embryonic development and transport, ovarian function and hormonal support of implantation, and uterine receptivity.

Usefulness of Results:

Effective female reproductive risk assessment has been encumbered on two fronts: 1) lack of information on the incidence and causes of VEP loss in humans, and 2) lack of data on homologous mechanisms of VEP loss in animals and humans. The products of this project will address both needs. By improving our ability to predict VEP loss in humans, based on the dose-response data obtained from animals, uncertainties associated

with assigning specific "margins of safety" should be reduced. Furthermore, this research will identify an array of endpoints with improved sensitivity and mechanistic value that may be added to the relatively insensitive fertility measures currently used in hazard identification. Such endpoints may be considered for inclusion into the EPA Female Reproductive Risk Assessment Guidelines.

Project Length and Cost: 3 yr FY89: $106K

Project Officer: A. Cummings (OHR/HERL-RTP) (919) 629-5194

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.1 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: Reproductive Toxicants and the Hormonal Control of Ovulation

Description: In the mammalian female, appropriate endocrine signals are essential for the production of gametes by the ovary. As spontaneous ovulators, both the human and rat exhibit regular ovarian cycles. In both species, the key endocrine event controlling ovulation is the appearance of the midcycle surge of luteinizing hormone (LH) from the pituitary into the general circulation. This clearly defined surge stimulates the meiotic and cytoplasmic maturation of the oocyte and triggers those follicular events that lead to the release of oocytes.

Various pharmacological manipulations (e.g. administration of the alpha-adrenergic blocking agent, phenozybenzamine) or exposure to substances of abuse (e.g. ethanol, tetrahydrocannabinol or morphine) can disrupt the surge and, as a consequence, block ovulation in several species including humans. Moreover, there is evidence (reviewed by Mattison, 1985) of menstrual alterations after occupational exposure to a variety of toxicants that may be linked to disruptions in pituitary regulation of ovarian function. Therefore, the proposed research will focus on the influence of environmental compounds on the hypothalamic-pituitary control of ovulation, an area in which there is a substantial data gap.

In the area of reproductive risk assessment, virtually no information is currently available on the effect of toxicants on the neuroendocrine control of ovulation. Since the key event in this process is the mid-cycle surge of luteinizing hormone (LH) from the pituitary gland, it is surprising that there is such a paucity of information because the surge is readily amenable to study. This is also disturbing, since an understanding of these effects is critical for the characterization of human risks associated with reports of menstrual irregularities or infertility in toxicant-exposed women. The proposed studies will evaluate the effect of selected environmental compounds on the ovulatory surge of LH in the rat. Since there is a large degree of homology between the rodent and human in the mechanisms controlling this event, and in the critical nature of the LH itself, the results of these studies will provide a valuable and important database for immediate use in risk assessment, as well as insight concerning potential effects of specified compounds on reproductive competence in the human female. The data obtained will provide definitive

information for use in reducing the current uncertainties associated with female reproductive risk assessment.

The proposed studies will employ an acute, in vivo administration of test compounds in different dose levels at specific times during the ovarian cycle and the identification of changes in LH secretion. Possible associated changes in other reproductive hormones will also be monitored. Initial selection of test agents will focus on those known to have adverse reproductive effects. This approach will provide a means to determine the way in which a compound affects the process of ovulation and the presence of any critical periods that may exist over the ovarian cycle during which an effect of exposure is more pronounced.

These studies will permit the identification of the immediate target tissue within the reproductive system (i.e., brain, pituitary or ovary). Initial experiments will investigate the effect of in vivo treatment on the LH surge. Once a compound is identified as having an adverse effect, subsequent in vitro experiments will examine potential impairments in hormonal release from hypothalamic and pituitary explants in order to determine the appropriate mechanism(s) of action. Furthermore, this research could lead to further studies assessing the impact of delays in ovulation on gamete viability, altered implantations, and possible abnormalities in fetal development that may result from changes in the critical timing of oocyte release.

Identified Results:

1. Recommended methods for the assessments of toxicant-induced disruptions in the ovulatory surge of luteinizing hormone

2. Report on the important temporal parameters associated with a compound's effect on the ovulatory surge of LH (and oocyte release) and the establishment of a critical database for such an effect

3. Report on the mechanisms underlying any observed alterations in the LH surge, using data collected from studies of hormonal release in vitro from pituitary and hypothalamic tissue

Usefulness of Results: Because the outlined approach will establish a much needed database for assessing the effect of environmental toxicants on gamete release, it is an important component of the study of female reproductive toxicology. Moreover, since these studies address the process of ovulation, as opposed to implantation and pregnancy maintenance, the data will permit an evaluation of a compound's effect on those events that occur prior to, and are necessary for, conception. Such information will fill a critical data gap that currently exists and reduce the attendant uncertainties associated with female reproductive risk assessment.

Mattison, D.R. Clinical manifestations of ovarian toxicity. In: R.L. Dixon (ed.), Reproductive Toxicology. Raven Pr., N.Y. (1985), pp. 109-130.

Project Length and Cost: 3 yr FY89: $131K

Project Officer: R. Cooper (OHR/HERL-RTP) (919) 541-4084

Topic: Biologically Based Dose-Response Models

Issue: 4.1.1 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: Neuronal and Hormonal Factors in Normal and Abnormal Kidney Development

Description: Deficits in the functional development of organ systems following in utero or early postnatal exposure to xenobiotic agents is one of the four manifestations of developmental toxicity. However, as noted in the Developmental Toxicity Risk Assessment Guidelines, relatively little attention has been paid to these adverse health effects. While some progress has been made in evaluating adverse health effects on the development of the central nervous system, a need exists to develop methodologies and evaluate the potential magnitude of effects for other systems. This project is an effort to expand our understanding of the pathogenesis of altered development of the kidneys. The expertise in physiological assessment of renal development available at HERL is being united with the expertise in biochemical and pharmacological development of the kidneys available at the Department of Pharmacology at Duke University Medical Center to provide an in-depth analysis of the issue.

This project will evaluate the hypothesis that disturbances in the development of either the endocrine or autonomic nervous system can cause altered morphological, biochemical, and physiological maturation of peripheral organ systems. That is, the very systems geared to maintaining homeostasis in the adult organism may also provide critical trophic influences on the development of organ systems in the perinatal animal. If true, the findings would demonstrate that the period of differentiation and activation of the autonomic these developmental events lie primarily in the third trimester. However, in the species used to evaluate potential developmental toxicity, they reside in the early postnatal period of development. Therefore, our present testing guidelines (which are focused on prenatal exposures and evaluations) would need to be and endocrine systems represent key critical periods in ontogeny. In the human, modified to accommodate them. In addition, since the effects on the organ systems would occur after the period of organogenesis, no gross morphological indications of the perturbation would be apparent. Thus, biochemical and physiological approaches are required to evaluate the hypothesis.

Because the events under study occur in the early postnatal phase of rodent development, easy experimental manipulation of the developmental processes involved is possible. To evaluate the hypothesis, three different sets of neuronal or hormonal perturbations will be employed: 1) global sympathectomy (via systemic administration of 6-hydroxydopamine) or central catecholaminergic lesioning via intracisternal administration) immediately after birth; 2) hyper- and hypo-thyroidism and exogenous glucocorticoid therapies (treatments known to influence the onset of sympathetic innervation); and 3) the effects of teratogens with potential action on the kidney mediated through the hormonal or neuronal alterations. For each of these models, four test batteries will be conducted that will establish neuronal status (degree of impulse flow, reflex stimulations, etc.), tissue responsiveness (renal cyclic ADH stimulation), and finally differentiation of renal tissue (nucleic acids, protein synthesis, ODC, polyamines and histology). The application of the test batteries to the experimental models will provide a comprehensive picture of how neuronal and hormonal input contribute to the development of renal structure and function and show conclusively whether teratogenic insult to the kidney may arise from primary insult to neuronal or hormonal systems.

Identified Results:

1. Compilation of reports detailing the effect of direct manipulation of the developing autonomic nervous system on the biochemical and physiological development of the kidney

2. Compilation of reports detailing the effect of manipulation of thyroid and adrenal function on the biochemical and physiological development of the kidney

3. Compilation of reports detailing the involvement of the neuronal and endocrine systems in chemically induced alterations in the functional development of the kidneys

Usefulness of Results: As stated in the Developmental Toxicity Risk Assessment Guidelines, the need exists to understand the potential of chemicals to affect the functional development of organ systems following exposure during the perinatal period. The risk assessor will benefit from this project in several ways: 1) by the project's focus on assessment of postnatal organ function and how that function can be permanently perturbed by exposure to xenobiotic agents at what may amount to be a new critical period; 2) the exposure being evaluated (the early neonatal period) is a time period in which little toxicological data is available; and 3) the mechanistic approach to determine how organ dysfunctions could arise in the absence of gross morphological disturbances. These items will provide important new information on how functional decrements in organ performance might be induced in developmental toxicity assays and whether our present testing strategies are adequate to detect these adverse effects.

Project Length and Cost: 3 yr FY89: $125K

Project Officer: R. Kavlock (OHR/HERL-RTP) (919) 541-2326

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.2 Inter/Intraspecies Extrapolation: Interspecies Sensitivities

Status: New

Title: Embryo Culture: A Unique in vitro Tool to Investigate Critical Issues in Interspecies Extrapolation in Teratology

Project Status: New

Description: Risk assessors in the area of developmental toxicity must often confront the wide disparity in reactivity of laboratory species to chemical exposure. In the absence of data to the contrary, the customary solution to this problem is to use the most sensitive species for establishing the NOEL. Uncertainties in the process would be reduced if the pharmacokinetic and/or pharmacodynamic factors responsible for these species differences were understood. Such knowledge would allow a more logical selection as to which species provides the most meaningful indicator of potential human risk.

The in vitro culture of embryos in co-culture with primary hepatocytes offers one approach to determining the relative contribution of metabolism and target tissue sensitivity in teratogenesis. Mouse, rat and hamster embryos can now be routinely

explanted from the uterus at the 4-12 somite stage and grown in culture. During the 48-hour period in which embryonic development is comparable to the in vivo situation, the embryos are isolated from the influence of maternal metabolism, kinetics, and placenta transport. Addition of the chemical to the culture medium allows direct determination of the effects of the agent on the embryo. Co-culturing primary hepatocytes with the embryo, in turn, facilitates understanding of the role of metabolism in teratogenesis (embryos of this developmental stage lack most Phase I metabolizing pathways). The hepatocytes need not be of the same strain or species as the embryo, and in practice, matrices of hepatocytes/embryo combinations can be constructed which can be used to contrast metabolic versus tissue sensitivity factors in the etiology of birth defects. Hepatocytes offer a major improvement over the widely used S-9 preparations as a metabolizing system, both in terms of duration and variety of metabolites produced. This co-culture system was developed and validated at the HERL laboratory. While not yet in use, human metabolic systems, derived from either placentas or liver biopsies, can be incorporated into the system. Results from this approach would enable direct comparison of interspecies metabolism in teratogenesis. Initial experiments will focus on chemicals which have been demonstrated to induce varying results in rodent/rabbit developmental toxicity studies. Likely candidates include endrin (a hamster but not mouse or rat teratogen); meclizine (a mouse but not rat teratogen), ethylenethiourea (a rat but not hamster, guinea pig or mouse teratogen), phenytoin (a strain-specific mouse teratogen), as well as several "unidentified" species-specific teratogens supplied by researchers in the pharmaceutical industry interested in evaluating the system.

Identified Results:

1. Reports describing quantitative dose-response information for heterologous hepatocyte/embryo combinations on a chemical by chemical basis. These reports will shed light on the relative contribution of metabolism versus embryonic sensitivity as explanations for observed strain or species differences in teratogenesis.

2. Summary evaluation of the use of the hepatocyte/embryo culture system in assisting selection of the most appropriate species for use in extrapolation to humans.

Usefulness of Results: The presence of marked species differences in the embryonic response to some environmental agents reduces the certainty of their respective risk assessments. For example, resolution of the potential human teratogenicity of the pesticide carbaryl has not occurred despite many years of debate within the Agency. For that chemical, the dog stands out from nearly ten other laboratory species as being a sensitive species. Whether the majority of negative species, or the reaction of the dog should be used to predict human risk has been subject of a yet unresolved debate. Similar, but less dramatic examples of species differences in teratogenic susceptibility are not rare. This project offers one possible approach to examining the underlying mechanisms behind these at times dramatic differences. The demonstration of either a unique species-specific metabolic pathway or a species-specific "embryonic receptor" responsible for such observations would be a major contribution in determining the most appropriate factors to consider in assessing human risk. While this project has certain limitations due to the limited number of species that can be cultured and the limited duration of the culture period itself, it does offer hope for determining the mechanism responsible for species differences in teratogenic susceptibility for at least some chemicals. As such, it is a potentially powerful tool for improving the scientific basis of risk assessments in the field of developmental toxicity.

Project Length and Cost: 3 yr FY89: $152K

Project Officer: J. Rogers (OHR/HERL-RTP) (919) 541-5177

Topic: Biologically Based Dose-Response Models

Issue: 4.1.2 Inter/Intraspecies Extrapolation: Interspecies Sensitivities

Status: New

Title: Utilization of Testicular Perfusion to Characterize the Effects of Leydig Cell Toxicants on Spermatogenesis in Several Species of Laboratory Animals

Description: The testis of the male is divided into two morphologically distinct compartments that are functionally dependent upon each other's hormonal secretions. Spermatogenesis (sperm production) occurs in the tubules of the testes, the first compartment, and this process is dependent upon testosterone that is secreted by the Leydig cells in the interstitial space between the tubules. Alterations of Leydig cell functioning may result in azoospermia and infertility. For example, spermatogenesis declines in aging men as the Leydig cell population falls below the threshold level necessary to maintain sperm production. The hypogonadal male, where the pituitary does not secrete the hormones (FSH and LH) essential for normal testicular function, is another example of the importance of Leydig cell function. These men have low sperm counts, in part, because in the absence of LH the Leydig cells do not secrete sufficient testosterone to maintain spermatogenesis.

The recognition of the critical relationship between intra-testicular testosterone levels and spermatogenesis has prompted the REAG/EPA to fund a study examining the decline in sperm counts in men following the administration of exogenous testosterone. High serum testosterone levels provide negative feedback in pituitary LH secretion with the subsequent decline in LH, Leydig cell function and spermatogenesis. The RTB/HERL plans to conduct a similar study using rats to that comparative dose-response relationships between administered testosterone and sperm production will the available. The present study will also examine the effects of Leydig cell toxicants (testosterone and EDS) on steroidogenesis in various mammalian species using the perfused whole testes from several species (rats, rabbits, and possible primates) following in vivo and in vitro administration of the compounds. The rabbit has several biochemical and morphological homologies with primates that the rat does not afford. Primate and human testes will be utilized if available. This task will also compare the effects of EDS administration in young, middle-aged and old male rats to determine if a safety factor of 10 for intraspecies variation in susceptibility is sufficient.

For these studies, whole testes will be perfused and the functional capacity of the Leydig cells will be evaluated using a variety of biochemical and morphological measures after both in vivo and in vitro EDS administration. In the in vivo studies spermatogenesis will be monitored by light microscopy, and daily sperm production, epididymal sperm reserves, and sperm morphology will be measured. In concert, the testes will also be examined and changes in Leydig cell numbers and stage-specific degeneration of the seminiferous epithelium will be quantified. In vitro the specific site of toxicant action in the Leydig cell will be determined using HPLC analysis of steroid substrate accumulation in the biosynthetic pathway.

Identified Results: This task will define the quantitative relationship between Leydig cell function and spermatogenesis in different mammalian species. The results of this research, in conjunction with other Agency studies (REAG study on the effects of testosterone on human spermatogenesis and a similar HERL study using rodents) will enable risk assessors to extrapolate effects on Leydig cell function, seen in toxicology studies using rats, to potential effects in man.

Usefulness of Results: This information will be useful to the Program Offices that receive reproductive data because it will enable them to determine if the effects on hormone synthesis, seen in rodents studies, will produce adverse effects on human sperm production, resulting in infertility in man. The database will be useful to REAG because it provides multiple reproductive endpoints across doses in two mammalian species.

Project Length and Cost: 3 yr FY89: $100K

Project Officer: John Laskey (OHR/HERL-RTP) (919) 541-2782

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.3 Inter/Intraspecies Extrapolation: Intraspecies Sensitivities

Status: Ongoing

Title: Mechanisms of Litter Effects: Assessing the Reliability of Dose-Response Models in Teratology

Description: A major problem in interspecies extrapolation of experimental teratology data to the human species is that rodents have many offspring per pregnancy, and the response among the fetuses in a litter can be quite variable. Since it is the pregnant animal, and not the fetuses, that is treated with the test compound, the individual fetuses cannot be treated as independent events statistically. Furthermore, it is often observed that the variation among the litter responses at a given dose is greater than one would expect from the variation of the responses within the litters. These issues become important in developing mathematical dose-response models. Is intralitter variability always less than interlitter variability? What are the biological bases for litter effects? Are these effects similar across compounds? It is possible to incorporate such so-called "litter effects" in statistical models of teratology data, but one needs to have an idea of how such effects occur, and how large one might expect them to be in our test species.

We will first study litter effects in the mouse using the known teratogen dinocap. The major malformation produced is cleft palate, and we will analyze the within-litter distribution of cleft palates following doses of dinocap. We will also record te position, weight, and sex of each fetus in the uterus. These data should allow us to measure correlations between fetuses in a litter, including within vs. between litter comparisons and analysis of "neighborhood effects" (whether neighboring fetuses in a litter respond more similarly to a toxicant than do non-neighboring fetuses). These results will provide information on how to incorporate overall litter effects into dose response models.

A possible cause of litter and neighborhood effects is variation among dams in the dose delivered to their uterus and to different pups within a uterus. In the second part of the study we will quantify pharmacokinetic variation by measuring the delivered dose of selected compounds to litters and to individual fetuses within litters. These measurements should allow us to quantify the contribution of pharmacokinetic variation to litter effect, both through variation in delivered dose and as a component of position and neighborhood effects.

A major criticism of all current models is the lack of incorporation of biological mechanisms responsible for intra- and interlitter variability. Any practical, biologically based dose-response model will have to address two problems: accounting for among-litter variability and predicting mean responses for any given dose. The results of these studies should contribute to the development of such models, with the aim of reducing the uncertainty involved in developmental toxicity risk assessment.

There are two components of this project. One is the actual animal and laboratory work involved in gathering the needed biological data. This effort will be supported by one NSI person. The second component is statistical design and mathematical modeling of the data. This work will be completed by a National Research Council research associate.

Identified Results:

1. Report on the prevalence of the "neighborhood effect", its magnitude, and its contribution to the litter effect
2. Report on the extent to which pharmacokinetic variation can contribute to the observed neighborhood and litter effects
3. Assessment of the value of, and methods for, incorporation of information from (1) and (2) above into practical, biologically based, dose response models for developmental toxicity data

Usefulness of Results: As indicated above, modeling of rodent developmental toxicity data is complicated by the fact that these species have multiple offspring per litter. Using a simple mean value to represent the response of a litter results in the loss of information concerning intralitter variation. This research will lay the groundwork for 1) assessing the impact of litter and neighborhood effects on variability seen in developmental toxicity data, and 2) developing methodologies for incorporating these variables into biologically based dose-response models.

Project Length and Cost: 3 yr FY89: $106K

Project Officer: J. Rogers (OHR/HERL-RTP) (919) 541-5177

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.3 Inter/Intraspecies Extrapolation: Intraspecies Sensitivities

Status: New

Title: Intraspecies Variability in Litter Responses to Developmental Toxicants: Role of Individual Variation in the Maternal Genome

Description: It has long been hypothesized that a significant component of intra-species variation in the maternal/litter response to teratogenic agents is due to individual genetic variation. This research project will evaluate the degree of individual variability resulting from exposure to a variety of teratogens administered at different times to outbred strains in two commonly used laboratory species (rat and mouse).

Standard teratology bioassays require that the maternal animal be killed shortly before term so that the fetuses can be removed for morphological examination. This procedure does not allow for any evaluation of the relationship of individual maternal variability (e.g., in pharmacological parameters) to developmental outcome. The proposed series of studies will directly address this critical issue by utilizing a strategy involving the repeated testing of a series of unrelated compounds known to induce developmental toxicity that may be ascertained by postnatal examination. Individual animals will be exposed to the same compound and dose during three successive pregnancies. Outbred female mice and rats will be bred to identified inbred males and teratogens administered at established effective doses. Data will be analyzed to determine the degree of individual constancy in teratogenic response across breedings; the relationships(s) of litter load and of maternal weight to fetal outcome; and the degree of similarity in the above factors for the different compounds and species tested.

Identified Results:

1. These studies will yield data sets that will allow analysis of the degree of intra-species variability that may be due to individual factors.

2. The above analysis should allow the empirical placement of confidence limits on the degree of inter-litter variation that can be ascribed to intra-species genetic variation.

3. The data generated in the studies should therefore allow regulatory personnel to investigate and/or improve the rationale for the "100-fold" safety factor often used in setting exposure limits for environmental agents. This safety factor assumes a 10-fold intra-species variability due to genetic differences, an assumption not firmly based on data generated in the test species employed in the laboratory.

4. Peer-reviewed publications on these studies, and the presentation of the data at scientific meetings.

Usefulness of Results: The extrapolation of developmental toxicity data generated in standard laboratory bioassays often involves the setting of safety factors. These, in turn, are based in large part, on presumed inter- and intra- species variability. Generally, factors of 10 are used for each of these sources of experimental variability. While the accumulated data from studies of developmental toxicity have allowed scientists to gain some insight into inter-species variability, the question of intra-species variability as it may impact responses to developmental toxicants has remained largely unanswered. The proposed studies will directly address this issue for its enlargement. Such data can then be utilized to justify and/or alter the safety factors employed to account for the component of variability due to individual genetic differences.

Project Length and Cost: 1 yr FY89: $66K (for NRC Fellow)

Project Officer: N. Chernoff (OHR/HERL-RTP) (919) 541-2651

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.1 Exposure Scenarios: Mechanisms Across Dose

Status: New

Title: Maternal Toxicity and Nonspecific Stress in Developmental Assessment

Description: EPA offices currently determine if a compound is a developmental toxicant by evaluating data that has been collected using standardized testing. These guidelines suggest multiple dose levels with the highest dose level being one that induces some form of overt maternal toxicity. Maternal toxicity is usually defined as weight loss or lethality. Uncertainty exists in the risk assessment of suspected developmental toxicants when fetotoxic effects occur only at levels producing an alteration in maternal health status. When agents are fetotoxic only at doses that are also maternally toxic, it is particularly difficult to extrapolate from high to low dose. Thus greater uncertainty exists in the ranking of these agents for selective developmental toxicity and for further toxicity testing when the Lowest Observed Effect Level is the same for the adult and the developing organism.

This uncertainty is partly due to the lack of understanding of the relationship between maternal and developmental toxicity. It is currently difficult to separate direct fetotoxic effects of an agent from those effects that are produced by an alteration in the maternal compartment. Current information is inadequate to assume that developmental effects at a maternally toxic dose only result from maternal toxicity. Such agents may in fact be directly active in both populations but be effective by different mechanisms. The uncertainty in risk assessment exists because there is no adequate database concerning the influence of maternal effects on the dose-response curve in the fetus. Because maternal toxicity is not usually an area of study in and of itself but is rather a component of the standard teratology protocol, there is little available data on the specific developmental effects associated with defined alterations in the maternal compartment. Thus, the lack of a database on the effect of maternal toxicity and/or nonspecific stress factors on the subsequent embryonic and postnatal development processes impedes the risk assessment decision-making process.

This project focuses on the maternal compartment and will provide data on the specific developmental effects associated with defined alterations in the maternal compartment. The health status of the dam will be altered in several ways, including the use of agents known to induce specific types of toxicity an/or conditions producing general or nonspecific stress. Concurrent controls will include non-pregnant animals and dams that are allowed to go to term for offspring evaluation. Biochemical, physiological, and functional indices will be used to evaluate the organ systems involved in maternal toxicity and nonspecific maternal stress. Collaborative portions of this project will include teratological and histological evaluations.

Identified Results:

1. Report that outlines the adverse developmental endpoints associated with specific maternal alterations

2. Report containing database to demonstrate the frequency of each category of adverse developmental effects resulting from maternally toxic and nontoxic doses of well-characterized doses

Usefulness of Results: The goal in dose-response assessment is to define the relationship of the dose of an agent and the occurrence of developmental toxic effects. This relationship is then used to extrapolate from high doses administered in experimental animals to the low exposure levels expected for human contact with the agent in the environment. As noted in the Developmental Risk Assessment Guidelines, it is difficult to correlate developmental effects with maternal toxicity, especially in the cases where developmental and maternal toxicity coexist. Information provided by this research will reduce the uncertainty associated with ranking agents for specific developmental toxicity and aid in the decision

of whether specific chemicals should undergo further testing. Future research approaches/strategies will be developed from the data produced by this project.

Project Length and Cost: 3 yr FY89: $71K

Project Officer: D. Miller (OHR/HERL-RTP) (919) 541-4186

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.1 Exposure Scenarios: Mechanisms Across Dose

Status: New

Title: Maternal Stress Response to Chemical Exposure: Impact on Zinc Status During Pregnancy

Description: General maternal stress, including that induced by toxic levels of chemicals, has been demonstrated to adversely affect fetal development. Further, there is evidence to suggest that stress affects zinc homeostasis. Zinc deficiency has been found to be teratogenic in all species where it has been investigated, including humans. These studies will investigate the relationships of zinc status, maternal stress response, and adverse developmental outcome in experimental animals. If maternal zinc homeostasis is impacted by the stress response, the zinc status of animals prior to and during pregnancy could be a critical factor in determining litter sensitivity. Components of compound-induced stress (i.e., corticosterone levels, induction of metallothionein synthesis, and effects on thymus, adrenal, spleen, and whole animal weights) will be measured following exposure of pregnant rats and mice to diverse chemical toxicants. The levels of stress will be correlated with the degree of developmental toxicity including effects on postnatal survival of offspring. Interactions of zinc status with chemical toxicity and/or stress will be assessed by measuring maternal and fetal tissue zinc concentrations in exposed and control animals fed dietary zinc at levels varying from deficient to supplemented. If significant interactions between compound-induced stress and zinc homeostasis are demonstrated, further work will define the most sensitive tissue and/or intracellular sites by following the absorption and distribution of ^{65}Zn. All of the trace element and metallothionein analyses, and a portion of the gross developmental toxicity studies, will be done at the Institution receiving the Cooperative Agreement.

Results:

1. Maternal/fetal tissue levels of zinc and developmental outcome following chemically-induced stress under conditions of varying dietary zinc levels
2. Data on effects of chemically induced stress on maternal organ weights and plasma corticosterone, and metallothionein concentrations in maternal and fetal tissues
3. Effects of chemically induced maternal stress on the absorption and distribution of Zn^{65}
4. Data derived from these studies will be presented at scientific meetings and published in appropriate toxicology journals

Usefulness of Results: The proposed research will yield data of value in two areas of regulatory toxicology. The first concerns the relationship(s) of zinc homeostasis, stress, and adverse developmental outcome in the laboratory setting. Standard teratology bioassays require exposures to test agents at levels producing maternal toxicity and attendant stress during pregnancy. Increased knowledge of the role(s) of zinc in the maternal and fetal responses to agent-induced stress will improve our ability to interpret data obtained at these high dose levels. The second area concerns the extrapolation of experimental data to humans. Zinc is an integral part of over 100 critical enzymes, and in the United States it is thought that a substantial percentage of women of childbearing age may not receive adequate levels of zinc in the diet.

Given these facts and the putative relationship between zinc status, stress, and developmental outcome, the proposed studies should generate data which will help define the role of stress and/or zinc deficiency in intraspecific variation in sensitivity to developmental toxicants. These data will therefore allow the Agency to consider dietary zinc levels in human subpopulations during the process of extrapolation and the determination of safety factors.

Project Length and Cost: 3 yr FY89: $75K

Project Officer: J. Rogers (OHR/HERL-RTP) (919) 541-5177

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.1 Exposure Scenarios: Mechanisms Across Dose

Status: New

Title: The Potential Existence of Low-Dose Thresholds for Developmental Toxicity: Quantification of Cell Death, Cell Cycle Perturbations, and Repair Capacity in the Mammalian Embryo

Description: To create biologically based dose response models for developmental toxicity risk assessments, knowledge is needed concerning the existence of doses below which biologically significant effects are not seen. The critical question of the possible existence of such threshold doses in teratology has never been answered and is essential for the development of biologically relevant mathematical models for low dose risk assessment by regulatory agencies. The proposed research will utilize novel approaches to determine if such thresholds exist for developmental toxicity. Historically, research efforts directed at this question have involved the use of extremely large numbers of animals to extend the dose-response curve to lower levels. Such "mega mouse" studies have not been successful since they have not addressed the central issue of the repair capacity of embryos following xenobiotic-induced damage.

The proposed research uses a combination of a sensitive assay for embryonic cell death using vital dye staining of whole embryos, flow cytometric cell cycle analysis, and a detailed analysis of the most sensitive target structure(s) of the fetus including gross examination, skeletal and cartilaginous analysis, and histopathology. These studies will initially examine the role of cell death and cell cycle perturbations in the developmental

toxicity of several classes of developmental toxicants. By using cell death and cell cycle effects as endpoints at multiple dose levels, we will be able to quantitate the repair capability of the developing conceptus. This will yield information on the presence of thresholds for different classes of teratogens. Studies done in collaboration with laboratories studying embryonic cell death and flow cytometric analysis of cell replication cycles have indicated that such an approach is viable. While HERL has standard teratology bioassay and flow cytometric capabilities, it lacks expertise in sensitive assays for the identification of embryonic cell death. The bioassay for cell death, and the detailed analysis of fetal morphology will be accomplished at the Institution receiving the Cooperative Agreement.

Identified Results:

1. A report on the effects of an alkylating agent (e.g. cyclophosphamide) on patterns of cell death, cell cycle alterations, and repair capability in the murine embryo at dose levels ranging from embryolethal to "sub-teratogenic."

2. Similar data on other classes of teratogens as that obtained with the alkylating agents. This body of data may enable evaluation of the general applicability of the threshold concept for developmental toxicity.

3. The data derived from these studies will be presented at scientific meetings and will be published in peer-reviewed toxicology journals.

4. The data will be presented to appropriate regulatory personnel in the Program Offices of the EPA in Washington, D.C. through seminars documenting the progress of this research effort.

Usefulness of Results: The quantitative risk assessment of developmental toxicants suffers from a lack of biologically valid mathematical models for extrapolation of laboratory data derived at high dose levels to the low dose levels relevant in environmental exposures. One of the major problems in the establishment and validation of such models is the question of the existence of threshold dose biological markers of toxic effects occurring at doses below those producing gross terata offers the most viable approach to the elucidation of low dose effects and/or the presence of threshold phenomena. The goal of the research is to establish whether thresholds for developmental toxicity are a general phenomenon. The conclusions of these studies will provide a biological basis for the inclusion or exclusion of the threshold concept in the construction of dose response models.

Project Length and Cost: 3 yr FY89: $50K

Project Officer: N. Chernoff (OHR/HERL-RTP) (919) 541-2651

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.1 Inter/Intraspecies Extrapolation: Homologous Models

Status: Ongoing

Title: The Relationship Between the Probability of Infertility in the Male Rodent and Ejaculated Sperm Counts

Description:

Reductions in sperm counts have been detected in workers exposed to environmental pollutants like DBCP following complaints of long-term infertility. However, the utility of routine on-site surveillance of ejaculated sperm counts (EJC) to determine if occupational exposure has produced adverse effects on sperm measures is limited, except in the most extreme cases like DBCP, because of the extreme variability in EJCs within and between individuals. It is therefore essential to test chemicals for their potential to induce adverse effects on sperm measures and fertility in laboratory species and to extrapolate such effects to man.

To reduce the uncertainty in extrapolating effects seen in rodents to man, data must be acquired on a common endpoint. In man the most common reproductive data available through the years has been on sperm counts from fertility clinics. In fact, sufficient data were available on sperm motility and there is a paucity of human data on other reproductive endpoints (testicular biopsy for example). However, in the rodent EJCs have been difficult to collect and analyze and, because of a lack of a common reproductive endpoint, uncertainty exists on how to extrapolate effects seen in rodents to the human. Recently, however, a few studies have described a methodology that surmounts the problem of collecting an ejaculate from the male rodent (Peitz and Olds-Clark, 1986; Zenick et al., 1984).

With these newly developed techniques, the relationship between the EJC of rodents will be compared with infertility. This project will define the relationship in rodents between EJC and infertility, compare the curves between the human and rodents, and determine the relative value of other endpoints (testis weights, serum hormones, histology, testicular sperm production, caudal sperm reserves, sperm morphology and motility). The EJC will be manipulated through direct alterations of CNS, hypothalamic, pituitary, or gonadal function. This information is currently unavailable and will enable us to provide quantitative estimates of uncertainty associated with rodent to human extrapolation in male reproductive toxicology.

Identified Results:

Short-Term - Development of the mathematical relationship between ejaculated sperm count and infertility in the rat using a model of congenital germ cell agenesis and comparison of this function with that described for humans.

Long-Term - Development of a critical database that compares the relationship between ejaculated sperm counts and infertility using chemical insults that target different male reproductive tissues. Determination of the mathematical relationship of other reproductive endpoints to the ejaculated sperm count and infertility.

Usefulness of Results:

The results of this task will be useful to both REAG/OHEA and the Program Offices. REAG will utilize this database to model the relationships between measures of target organ impairment and reduced reproductive success. Using the database developed in the current task, the Program Offices will be able to predict the adverse effect on fertility from changes seen in other reproductive endpoints. This is important because the Program Offices often receive data indicative of reproductive alterations in the absence of a fertility assessment.

Project Length and Cost:

3 yr FY89: $56K

Project Officer:

E. Gray (OHR/HERL-RTP) (919) 541-7750

D.3 PULMONARY

By virtue of its functional intimacy with air and its varied constituents, the lung is a target and/or conduit for potentially toxic inhalants. Although our concern resides in human health, the bulk of existing toxicology that are acute or short-term. Hence, there are at least two major uncertainties in our understanding of inhalant toxicity: the dosimetric/extrapolative linkage between experimental animals and man, and the inter-relationship between exposure and duration in the elicitation of response. The overall objective of this initial program is to establish a framework that has some general applicability to these issues. The projects being initiated will not produce final or universal answers, but will be systematic studies with these issues as primary hypotheses rather than retrospective attempts to coalesce the data in a poorly thought through context.

Ozone is one of a few toxicants for which there is multi-species and multi-endpoint toxicology and for which it is also possible to do experimental human studies. With the aid of a novel nonradioactive tagging procedure, it is possible to acquire data on tissue and cell target dose which can be correlated with biologic effect. Obviously, more invasive detail can be obtained in animals and at present considerable data exist for the rat. Limited studies are to be conducted in humans that can address lavagable cell dose data which can be related to inflammation and immune function. Nonhuman primates offer the opportunity to study dose/effects using identical techniques and permits invasive assay of endpoints as in the rat. Moreover, the larger size of the animal, not to mention the similarity to man in lung structure, increase both the sensitivity and precision of the isotope analysis. Collectively, the diversity of exposure across these animal species, the dosimetry which includes man though in a more limited fashion, and detailed cell biology assay will allow construction of a credible model of lung injury which has a cross-species dose and response framework for the purpose of extrapolation and risk assessment.

The inter-relationship of concentration and time is particularly important to the risk assessment process. This relationship is especially critical to the RfD process, which attempts to extrapolate over lifetime periods. Recently, the assessment of response over shorter periods as might be incidentally encountered has drawn more attention. The somewhat fragmented existing data suggest that, in the induction of lung injury, concentration dominates time in very acute periods. However, as concentration falls and time of exposure is extended, a more complex interaction function is observed, at least in preliminary work with ozone. Does this relationship hold with other air toxicants? endpoints? and over what periods? Can we develop a mathematical function that can be incorporated into the risk assessment process which can include concentration/time relationships?

Topic: Biologically Based Dose-Response Models

Issue: 4.1.2 Inter/Intraspecies Extrapolation: Interspecies Sensitivities

Status: New

Title: The Use of Inflammatory and Immune Response Indicators to Study Exposure-Response Relationships and Animal-to-Human Extrapolations

Description: This project will provide information regarding the effects of pollutants on hose defense systems in humans. It is intended to increase our understanding of exposure-response relationships and animal-to-human extrapolation as they relate to host defense systems. The response of similar target tissues in humans and rodents to chemical insults will be determined. Tissues from rodents and humans will be exposed in both in vivo and in vitro settings. Cellular, biochemical, and molecular changes will be compared. Specifically, this effort will examine the effects of pollutants on human and animal

immune cells with particular focus on pulmonary alveolar macrophages. These cells are the body's first line of defense against inhaled pollutants and microorganisms. Macrophages also produce enzymes that can lead to inflammation in the lungs, as well as tissue destruction. Thus, any adverse effect of pollutants on macrophages could lead to an increased susceptibility to infection as well as respiratory problems. To achieve the goals of exposure-response relationships and animal-to-human extrapolation as they relate to hose defense systems, exposures to pollutants of interest will be performed in animals and humans under controlled chamber conditions as well as in culture using human and animal inflammatory and immune cell types.

Identified Results: Comparative data on the effects of pollutants on human and animal host defense systems for risk assessors to judge differences in species sensitivity.

Usefulness of Results: Because host defense effects are often among the most sensitive effects examined in risk assessments, the results of this project will help reduce uncertainties in an important endpoint (species sensitivity). The complementary research conducted in animals and humans, in vivo and in vitro using sensitive biological endpoints relevant to hose defense mechanisms, will result in a better assessment of potential health effects associated with exposure to pollutants.

Project Length and Cost: 3 yr FY89: $171K

Project Officer: H.S. Koren (OHR/HERL-RTP) (919) 966-6254

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.2/4.1.3 Inter/Intraspecies Extrapolation: Inter/Intraspecies Sensitivities

Status: Ongoing

Title: Establishment of Intraspecies and Interspecies Linkages Between Delivered Dose and Toxic Effect: A Human/Monkey/Rat Comparison Using a Prototype Inhalant, Ozone

Description: This project will determine the relative responsiveness (sensitivity) of humans versus monkeys and rodents by correlating markers of both dose and toxic response in the three species. There are few toxicants for which a large multi-species and multi-endpoint toxicology database exists, and which can at the same time be feasibly studied in human clinical or epidemiological studies or in all species of laboratory animal. Ozone, which is such a toxicant, is being studied as a prototype inhaled pollutant in both human clinical studies and rat studies now in progress at EPA, Research Triangle Park. Techniques recently developed by EPA are being used which determine the dose of ozone (using oxygen-18 labeling techniques) delivered to pulmonary cells obtained by bronchoalveolar lavage. This measurement of dose is being correlated with quantitative immunological, biochemical, morphological, and molecular markers of toxicity. The ratio of delivered dose to toxic effect observed is interpreted as the "sensitivity" to ozone of a given cell or tissue.

The proposed cooperative agreement will provide data from studies with nonhuman primates that will be complementary to the rodent and human data. The addition of the

nonhuman primate data will add important morphological data on the airways and lung parenchyma that cannot be obtained with humans or rodents because of the invasiveness of scale problems. The availability of pulmonary lavage techniques makes it possible to sample human and animal cells which have been exposed in vivo to ozone. Using new labeling technologies, it will be possible to evaluate both the relative does and the sensitivity of different species to ozone, and by analogy, to other inhaled pollutants. The endpoints chosen will provide information on a broad spectrum of adverse health effects, including impairment of host defense to microorganisms, as well as reversible and chronic pulmonary health effects.

Identified Results: July 1990: Report entitled "Tissue dose effect studies of ozone in humans, rhesus monkeys and Fisher rats: implications for human risk assessments of inhaled reactive gases."

Usefulness of Results: This project will establish for the first time a link between human clinical studies data, nonhuman primate data, and rat toxicology data. It will do this at the tissue level, i.e., using measurements of the dose actually delivered to tissue and a multiplicity of toxic response measurements. The animal data are essential to provide an understanding of the full array of health effects of toxicants; however, the linkage of dose and sensitivity to the human must be made in order to accurately assess risk in humans. Questions about the applicability of rat data, or nonhuman primate data to the human, will be answered by this project.

Project Length and Cost: 2 yr FY89: $75K

Possible Grantees: Dr. Charles Plopper, Dr. Dallas Hyde, Dr. Jerold, Last School of Veterinary Medicine, University of California Davis, CA 95616; Dr. John R. Harkema, Lovelace Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185

Project Officer: G. Hatch (OHR/HERL-RTP) (919) 541-2658

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.1 Exposure Scenarios: Mechanisms Across Dose

Status: Ongoing

Title: A Lung Injury Dose/Time-Response Model for Assessing Toxic Inhalants

Description: A prototypic mathematical model initiated by OAQPS in an attempt to integrate data from human phosgene exposures with animal data on BAL protein for risk assessment purposes is replete with scientific and ethical uncertainties. Recently, another model describing the CxT relationships of ozone on BAL protein has also been proposed but lacks through concentration-time dependency information. A theoretical model needs to be integrated and strengthened by mechanistic data to become scientifically acceptable for prediction of human risk from combined animal and human data. The generation of validated models describing high dose/low dose relationships in animals would be amenable to critical study in the area of pulmonary toxicity. Pulmonary edema, caused by leakage of plasma from the blood into the air spaces, is sensitively and quantitatively detectable after

high as well as low levels of acute pulmonary toxicant challenge. Hence, accumulation of protein in response to damage and its disappearance, as is found in repair, could provide a credible basis for addressing "generic" lung injury and its repair. Protein in BAL of human subjects after exposure to oxidants has been reported and the kinetics of leakage appear to be quite similar to those described in animals. The model developed could provide a bioassay and extrapolative tool for risk assessment of noncarcinogenic substances.

A lung injury dose-response model will be generated that is applicable to pulmonary toxicity which permits intraspecies extrapolation between high and low dose and between single and repeated exposures. The model would have particular utility in addressing the poorly understood issue of acute to chronic relationships of toxicant lung damage and would also provide a means to generically examine a common index of lung damage likely with all inhalants.

From a matrix exposure design derived from existing preliminary data, a mathematical model will be developed which describes empirically the kinetics and CxT relationships of lung bronchoalveolar lavage fluid (BAL) protein accumulation, an indicator of alveolar-capillary permeability. The application of the model will then be explored by quantification of the deviations between expected and measured values under various CxT exposure scenarios. Rat data will form the basis of the model; the general applicability of the model will also be tested in other species. Other ongoing studies which employ C1C4C-phosgene and C1C80-ozone will examine whether using the "retained dose" rather than the "exposed dose" of toxicant will enable "normalization" of interspecies variations. While the empirical model is being validated, mechanistic experiments will be performed for the purpose of extending the model from a strictly empirical one to one which explains permeability changes in theoretical terms. Questions that will be addressed include: 1) "What is the potential influence of the protein turnover in the alveoli on the toxicity measurements?" 2) "What molecular probes provide optimal detection of alveolar permeability changes?" and 3) "Does repeated exposure reduce the sensitivity to permeability changes and does this alter is utility for predicting chronic disease?"

Identified Results:

1. Report on the mathematical relation between exposure concentration, duration, and time post exposure which will be validated for two inhaled toxicants. The model's accuracy in predicting measurements will be quantified in rats and spot-tested in three other species.

2. A report describing the feasibility of normalizing dose-response using "retained dose." The limitations of the existing model for risk assessments for phosgene as well as its refinement criteria will be included.

3. A description of analogies between criteria pollutants (ozone and nitrogen dioxide) and phosgene, and quantitative comparison of other toxicity endpoints (quantitative morphology, physiology, etc.) with the measurement of BAL protein. Such comparisons will test the broad applicability of the model.

4. A report describing the utility of the model for studies of complex atmospheres and varied exposure scenarios. Repeated exposure scenarios will be studied using the model to predict cumulative damage, and experiments will be planned to investigate the linkage between acute and chronic exposures.

Usefulness of Results: The generation of a validated model describing high dose/low dose relationships in animals would be amenable to critical study in the area of pulmonary toxicity. Such a model would assist OAQPS in problems of extrapolating between animals and humans,

between acute exposures of different lengths and conditions, and between other endpoints of toxic pulmonary response. The model would be applicable to Criteria or Air Toxic pollutants, both gaseous and particulate, which cause acute pulmonary toxicity. This model will benefit any Program Office in need of data and/or models of lung toxicity for regulatory processes. The RfD process would be greatly advanced with this model and the minimal data ultimately needed to utilize it for a vast array of potentially toxic substances to humans.

Project Length and Cost: 2 yr FY89: $256K*

*Costs include inhalation engineering support.
Costs include technical biological support to conduct project.

Project Officer: G. Hatch (OHR/HERL-RTP) (919) 541-2658

D.4 GENETIC TOXICOLOGY

The principles and concepts applied to cancer risk assessment are discussed in the 1986 "Guidelines for Carcinogen Risk Assessment" (which are currently being updated). These "Guidelines" point out major areas of uncertainty encountered during the assessment of human risk from exposure to environmental agents. Because cancer is an endpoint of major concern in regulatory decisions, the uncertainties associated with cancer risk characterization are important problematic issues that need to be addressed in a well-designed research program. The research areas of focus are in accordance with the issues described in Topic 4 and concentrate on the development of biologically based dose-response models for high-to-low dose extrapolation and response equivalence across species. These research needs relate to Issues 4.1: Inter/Intraspecies Extrapolation and 4.3: Mechanistic Variation, and contain aspects of Issue 4.2: Exposure Scenarios with respect to mechanisms across dose and sensitivity of endpoints as a function of dose.

A major uncertainty encountered in the risk assessment process concerns the validity of using the linearized multistage model (a curve-fitting approach) for high dose-to-low dose extrapolation. A focus of research in this area should be on the development of alternative mathematical models that incorporate information on key biologic processes and endpoints currently thought to be involved in carcinogenesis as opposed to statistical curve fitting. As pointed out in the "1986 Guidelines," progress in this area is dependent upon a better understanding of the underlying mechanisms that lead to neoplasia. Of major importance in this respect is a better understanding of those components of the cancer process, initiation, promotion, and progression, which are universally critical to the development of neoplasia, irrespective of the particular carcinogenic agent.

Although the mechanism of action of chemical and physical carcinogens is not known with certainty, almost all human carcinogens that have been examined to date appear to be mutagenic or reactive with DNA. It is this characteristic that currently permits EPA's use of inferences to predict carcinogenic activity. There remains a subset of carcinogens that do not appear to be DNA-reactive, however. This class, the so-called "nongenotoxic" carcinogens, presents an added uncertainty with respect to appropriate risk assessment models because they may not be best described by linear, nonthreshold low-dose multistage models. In addition, there exist agents that are not by themselves carcinogenic but can significantly enhance the yield of tumors or accelerate or modulate the progression of premalignant to malignant lesions (ie.., tumor promoters) or the biological characteristics of malignant cells (progressors). Very little information exists on the mechanism of action of "nongenotoxic" carcinogens. In some cases, compounds (e.g., amitrol activated by peroxidative systems) thought to be nongenotoxic have been shown to possess DNA-damaging activity. In other cases, nongenotoxic carcinogens will alter genetic material through indirect or non-DNA reaction mechanisms (e.g., physical interference by asbestos in chromosom-

al segregation, modification by 5-azacytidine of pathways involved in hypomethylation of DNA).

While definitive identification of specific steps in carcinogenesis will remain elusive, a deliberate research focus is proposed on the 1) identification of intermediate biological endpoints of carcinogenesis, 2) the generation of data on these endpoints, and 3) the application of this information for the development of biologically based mathematical models for low-dose extrapolation. Research efforts should concentrate on non-DNA reaction pathways or possible indirect pathways by which target gene expression (especially oncogenes and tumor suppressor genes) is changed. It should be emphasized, however, the generation of data is not necessarily a prerequisite for mathematical model development; it is recognized that model development may, in fact, help define important parameters.

Risk assessment for carcinogens is a complex process that relies on experimental data from animals and where possible on human cancer epidemiologic studies. It is frequently necessary to assume equal sensitivity for tumor induction in rodents and humans. Hence, another Topic 4 issue is the uncertainty surrounding species-to-species sensitivity extrapolation. Extrapolation of data from animals to humans with a reasonable level of confidence requires that the mechanism of action of carcinogens and the carcinogenesis process in humans and experimental species be similar. Some of the essential characteristics of the process of carcinogenesis can probably be determined by modeling and comparing the time course, dose-response, proliferation of stem cells and their predecessors, and the kinetics of their differentiation in relevant animal models as compared to what is known about the incidence and time course of human cancers. The testing of the assumption that humans are as sensitive as experimental animals to the chemical induction of tumors can also be investigated by techniques measuring certain genotoxic endpoints (e.g., methods to detect gene mutations in lymphocytes, cytogenetic techniques measuring sister chromatid exchanges, chromosomal aberrations including micronuclei) which can be evaluated in both experimental animal models and humans.

Topic: Biologically Based Dose-Response Models

Issue: 4.2.2 Exposure Scenarios: Sensitivity of Endpoints as a Function of Dose

Status: Ongoing OHEA/RURA project

Title: The Quantitative Relationship Between DNA Alkylations and Stable Chromosomal Damage

Description: For exposed populations of animals or humans, the level of DNA and nuclear protein adducts and chromosome aberrations induced in peripheral blood lymphocytes (PBL) and the level of formation of stable translocations induced in germ cells should each have a direct quantitative relationship to the level of EtO exposure. Quantitative data correlating these three endpoints—levels of DNA or protein alkylations, initial levels of chromosome aberrations, and levels of induced stable chromosomal rearrangements following exposure to various levels of EtO in any single test system—is not available. This project proposes to quantitative the dose and dose-rate relationship between EtO exposure, the formation of nuclear DNA and protein alkylations, the formation of chromosome aberrations at first metaphase, and the induction of stable translocations at the 5th population doubling level in the human fibroblast cell line IMR-90. If the quantitative relationships observed in IMR-90 cells are the same or similar to those in PBL and germ cells and if these relationships are similar for mice and humans, the data generated in this study will aid in assessing the risk of heritable DNA damage following the demonstration of a given level of either DNA or protein adducts or chromosome aberrations in PBL in EtO-exposed human populations.

Usefulness of Results: The proposed research project with Coriell Institute is designed to contribute to EPA's efforts in developing biologically based dose response models for health risk assessment by aiding in our understanding of the underlying causes of toxicity. Such biologically reflective risk models will provide more meaningful estimates of health risk associated with exposure to a toxic chemical. Ethylene oxide (EtO) was chosen for these studies because its physical/chemical properties and metabolism are relatively well understood. Moreover, there is a large database on the mutagenicity of EtO, including heritable germ cell chromosome translocations in mice and information concerning its ability to bind to or alkylate DNA and protein in animals and humans. The work at Coriell will aid in our efforts to develop a mathematical model for assessing risk associated with chromosome translocations. Chromosomal translocations have been associated with infertility, cancer, and other medical disorders.

Project Length and Cost: FY89: $95K

Project Officer: V. Dellarco (OHEA/GTAB) (202) 382-7332

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Topic: Biologically Based Dose-Response Models

Issue: 4.3 Mechanistic Variation

Status: Ongoing OHEA/RURA project

Title: A Class of Biologically Based Dose-Response Models

Description: The project is designed to develop a BB-DR model that includes the Moolgavkar and Venson (1979) model as a special case. The Moolgavkar and Venson model makes some undesirable biological assumptions and may not be applicable to data from animal bioassays that are used in cancer risk assessment. The mathematical model which requires numerical solution for some equations has already been developed. Our current effort is to develop a numerical algorithm to solve equations and to develop a computer software for constructing dose-response models.

Identified Results:

1. A document describing mathematical derivation of the model and procedure for using the computer software to construct BB-DR models
2. Computer software
3. Results will be submitted for publication

Usefulness of Results: The BB-DR model and computer software developed from this research will enhance CAG's ability in cancer risk assessment.

Project Length and Cost: 2 yr FY89: $75K

Project Officer: C. Chen (OHEA/CAG) (202) 382-5719

Topic: Biologically Based Dose-Response Models

Issue: 4.1.3 Inter/Intraspecies Extrapolation: Intraspecies Sensitivities

Status: Ongoing OHEA/RURA project

Title: Cohort Survivorship Analysis

Description: In retrospective/prospective cohort studies, large deficits of mortality will often be seen after the completion of an analytical phase. Several researchers have tried to explain these deficits by citing the healthy worker effect and preferential survivorship. Others have developed differing opinions regarding the reasons for these deficits and how these effects alter the direction of the differences, e.g., lengthy employment results in an "excess" of mortality. This study will utilize well-documented occupational cohorts that have been detected health effects. The beginning and ending period of follow-up of the study cohort will be represented by times A and B, respectively. A time Z will be chosen to represent a point in time during this period, such that A<Z<B. Members of this study cohort will be divided into unique subgroups. Persons hired during the period A to Z will be evaluated separately from persons hired during the period Z through B. Additionally, the mortality experiences of person hired during the period A through Z but who worked through Z will be examined separately from those who were hired during period A through Z but who were terminated prior to Z. The mortality experiences of all subgroups will be compared to evaluate the impact of the different potential biases alluded to earlier. The life-table analyses will yield observed and expected mortality bycause-specific categories by latent period and by length of exposure. Estimates of risks will be calculated based on these numbers.

Identified Results: Analyses that will provide evidence to support (or refute) the explanations given for the extreme, otherwise inexplicable, deficits (or excesses) often seen in occupational cohort studies.

Usefulness of Results: This information will be used to better interpret the results of occupational cohort studies for weight of evidence judgments.

Project Length and Cost: FY89: $40K

Project Officer: D. Bayliss (OHEA/CAG) (202) 382-5726

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Topic: Biologically Based Dose-Response Models

Issue: 4.3 Mechanistic Variation

Status: New RIHRA project

Title: Molecular Mechanisms of Initiation and Promotion

Description: This project will develop and characterize a new-generation in vitro mammalian cell transformation system that can be used to define, at the molecular level, the cellular site(s) of carcinogen-induced damage. This system is based on observations that at least one

member (alkaline phosphatase), and possibly others, of a class of unique cell surface proteins is lost from prospective tumor cells during the preneoplastic phase of the cancer process. The carcinogen-induced loss of this marker protein has been shown to occur across species (rats, mice, dogs, and humans) and carcinogen classes (nitrosamines, furans, and 2AAF). Because the new system duplicates these observations in vitro and focuses on a specific molecular event—the loss of a marker protein—it provides the opportunity to study the early actions of initiators and promotors at the molecular level under carefully controlled conditions.

Identified Results: This study will provide insights into initiation and promotion mechanisms and thus help to distinguish these two important components of the cancer process. It also provides an in vitro surrogate for the cancer process in animal species and humans.

Usefulness of Results: These products will directly aid in developing biologically based dose-response models by providing data for better distinguishing between initiators and promotors. Equally important, the system opens up the possibility of pursuing modeling using in vitro methodologies.

Project Length and Cost: 2 yr FY89: $30K

Project Officer: D. Reese (OHEA/GTAB) (202) 382-7342

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Topic: Biologically Based Dose-Response Models

Issue: 4.3 Mechanistic Variation

Status: New RIHRA project

Title: Biologically Based Dose-Response Modeling for Chromosome Translocation—An important element in cancer and genetic risk assessments

Description: The objectives of this research are:

1. To construct a biologically based dose-response (BB-DR) model for heritable chromosome translocation. The model will be applied to data of heritable translocation induced by ethylene oxide (EtO) which is part of the EPA's ongoing effort on genetic risk assessment.

2. The mathematical formulation on the chromosome translocation will also be utilized to construct a cancer BB-DR model which assumes that the chromosome translocation is an initiation event in the context of initiation/promotion/progression carcinogenesis. This research supports the EPA's ongoing effort on arsenic risk assessment (please see the attachment) and BB-DR modeling in general.

3. To develop numerical algorithms and computer software for model construction.

Identified Results:

1. A documentation of mathematical derivation of model and procedure for using the computer software

2. A computer software for model construction

3. Results that will be submitted for publication

Usefulness of Results: Chromosome translocation is an important component in cancer and genetic risk assessments. The proposed research is the first attempt to construct a BB-DR model for chromosome translocation. The product will enable OHEA to construct a biologically based dose-response model on heritable translocation and will enhance OHEA's existing ability in cancer risk assessment.

Project Length and Cost: 2 yr $80K/yr

Project Officer: C. Chen (OHEA/CAG) (202) 382-5719

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Topic: Biologically Based Dose-Response Models

Issue: 4.3 Mechanistic Variation

Status: Ongoing OHEA/RURA project

Title: Generalized Dose-Response Model for Tumor Promoters Initiators

Description: This project focuses on developing and testing a generalized dose-response model for carcinogens which act as promotors, especially liver tumor promotors, since more nongenotoxic agents which EPA regulates as carcinogens produce liver tumors in animals. Included in this development is a method which incorporates the results from initiation-promotion assays into the generalized MoolgavKar-Venzon-Knudson model. This model also allows risk extrapolation for carcinogens which possess both initiating and promoting capabilities.

Identified Results: Products will be a new tested model for quantitative risk assessment which incorporates the mechanism of initiation-promotion. There will also be a computer program to solve for the necessary parameters and low dose risk estimates.

Usefulness of Results: Two advances here are: 1) use of cancer models which allow for various nongenotoxic mechanisms for carcinogenesis and 2) use of results from initiation-promotion shorter term assays in quantitative risk estimation.

Project Length and Cost: 3 yr FY89: $105K

Project Officer: S. Bayard (OHEA/CAG) (202) 382-5722

Topic: Biologically Based Dose Response Modelling

Issue: Cross-Issue

Status: Ongoing

Title: Improved Methods for Combination of Quantitative Risk Estimates for Carcinogens

Description: The goal of this work is to provide methods for incorporating a wide range of information into Agency carcinogen risk assessments. To date, a variety of methods have been rather inconsistently applied in those instances wherein there exists more than one data set suitable as the basis for a quantitative estimate. These have included choosing the highest quantitative estimate, rarely combining data prior to calculation of an estimate, and combining estimates across studies, across sexes or across species. This project has had a biological and a mathematical focus. The first step has been to examine existing carcinogen risk assessments for those wherein this was an option for combined risk estimation, cataloging the combination methods and identifying rationales for combining or choosing a single estimate. This has been followed by an effort to evaluate sources of valuability in data sets and evaluating risk estimates both from a biological as well as from a statistical standpoint. Concurrently, there is taking place an analysis of methods for preparing combined risk estimates; for example, the theoretical appropriateness of taking geometric means of upper limits values resulting from application of a linearized mutltistage procedure. Another approach being investigated is the application of statistical tests for validity of combining data sets prior to application of an extrapolation model. This work is proceeding through use of both extramural resources and Agency scientists and mathematicians. A workshop on results is planned for Spring of 1990.

Identified Results: Specific guidance on use of multiple data sets for quantitative risk assessments for carcinogens in the form of reports and published manuscripts. It is expected that results of this work will directly impact revisions to the Guidelines for Risk Assessment of Carcinogens.

Usefulness of Results: Carcinogen risk assessments based on all applicable data will warrant more confidence by risk assessment specialists and be better able to stand the test of public opinion and the courts. Guidance as to validity of combination methods is needed by ORD and program offices to produce such estimates.

Project Length and Cost: 3 yr FY89: $40K

Project Officer: R. Schoeny (OHEA/ECAO-Cin) (513) 456-7544
W. Farland (OHEA) (202) 382-7315

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.2 Inter/Intraspecies Extrapolation: Interspecies Sensitivities

Status: Ongoing

Title: Determination of Relative Rodent-Human Interspecies Sensitivities to Chemical Carcinogens/Mutagens

Description: The effect of chemical exposures will be measured by several complementary methods using a combination of morphological, genetic, cytogenic, enzymatic, and molecular biological markers. Detailed dose-response relationships will be modeled according to biological and statistical concepts to obtain the desired extrapolation (sensitivity) constants.

New molecular methods will be utilized to understand the fundamental interspecies differences between the response of rodent and human cells to genotoxic agents. A systematic evaluation of genotoxic responses will allow us to determine how genotoxic effects in rodents extrapolate to similar effects in humans. Research has already indicated that human cells may be more capable than rodent cells of repairing at least some DNA lesions, implying that human cells may be less sensitive to genotoxic agents. Molecular techniques (i.e., Insertion of human DNA repair genes and specific target genes) will be used to define, quantify, and understand the mechanisms of the specific interspecies differences. Such techniques have already proven genotoxicity. Quantitating these differences 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.

One of the major assumptions in the cancer risk assessment process is the assumption that humans will respond to the same extent as do rodents to the carcinogenic effects of chemicals (i.e., equivalent tumor incidence, tumor multiplicity, and tumor latency at equivalent doses). The uncertainty in the risk assessment process created by this assumption can be quite large ranging up to several orders of magnitude. A research program aimed at reducing this uncertainty by defining the relative response characteristics of similar tissues from both rodent and human sources for different classes of chemicals will help to reduce the uncertainty associated with this assumption and its detrimental effects on the risk assessment process.

Identified Results: Deliverable Title: Report and journal article defining potential fundamental mechanistic differences in the comparative response of rodent and human cells to genotoxic agents.
Deliverable date: 9/90

Deliverable Title: Report and journal article defining rodent cell test models which have been modified (i.e., by the addition of human DNA repair genes and/or metabolic activation genes) to make suitable for mechanistic comparison with human cells.
Deliverable Date: 7/92

Deliverable Title: Report and journal article defining species sensitivity constants for specific chemical exposure to rodent and human cells.
Deliverable Date: 8/92

Usefulness of Results: This research is directly applicable to the RIHRA process. The agency currently uses the assumption that humans will respond to the same extent as do rodents to the carcinogenic/mutagenic effects of chemicals. These assumptions include equivalent tumor incidence, tumor multiplicity, and tumor latency at equivalent doses. The uncertainty in this assumption can make the ultimate risk assessment incorrect up to several orders of magnitude. By comparing the genotoxic response characteristics of similar rodent and human tissues we can determine for particular classes of chemicals and particular types of genetic events (of the type associated with malignancy) the species sensitivity

constants. These interspecies sensitivity constants will provide information directly applicable to the risk assessment process.

Project Length and Cost: 3 yr FY89: $136K

Project Officer: S. Nesnow (OHR/HERL-RTP) (919) 541-3847

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.2 Inter/Intraspecies Extrapolation: Interspecies Sensitivities

Status: Ongoing

Title: Dose-Response Relationships for Human Gene Mutation

Description: The objective of this Cooperative Agreement is to compare the ability of human X-linked and autosomal genes to quantitative genotoxic damage from chemicals which are clastogenic (break chromosomes). This information will then be evaluated quantitatively with data from the same genes in rodents to assess relative rodent/human responses to genotoxic agents.

The major histocompatibility complex (HLA) of chromosome 6 will be developed for use as a marker for induced genotoxic damage. A selection system for HLA mutants will be analyzed. When the HLA system is developed, the ability of the HLA markers to detect clastogenic damage will be evaluated and compared to that of the hgprt locus. This comparison will include the relative magnitude of the induced frequency as well as karyotypic and molecular analysis of mutants. The response of human cells to genotoxic agents will be compared to that obtained in the currently used rodent cell systems.

This basic information concerning the gene mutation response in rodent and human cells will be combined with other aspects of the rodent-human interspecies response to genotoxic agents project. The information gained in the integrated project will allow the determination of sensitivity constants for the rodent and human cells. This comparison will provide information concerning the relative sensitivity of rodent and human cells to carcinogenic/mutagenic chemicals.

One of the major assumptions in the risk assessment process is that humans will respond to the same extent as rodents to the carcinogenic effects of chemicals. A systematic evaluation of genotoxic response (including specific gene loci) will allow us to determine how genotoxic effects in rodents extrapolate to similar effects in humans. This Cooperative Agreement will develop the genetic markers and the comparative marker results necessary to evaluate genotoxic effects in humans.

Identified Results: Deliverable Title: Determination of dose-response relationships for human cell mutation using the HLA and HGPRT markers.
Report and Journal Article
Deliverable Date: 9/91

Deliverable Title: Comparison of rodent and human gene mutation response to specific classes of chemicals
Report and Journal Article
Deliverable Date: 2/92

Usefulness of Results: This research is directly applicable to the RIHRA process. The Agency currently uses the assumption that humans will respond to the same extent as do rodents to the carcinogenic/mutagenic effects of chemicals. These assumptions include equivalent tumor incidence, tumor multiplicity, and tumor latency at equivalent doses. The uncertainty in this assumption can make the ultimate risk assessment incorrect up to several orders of magnitude. By comparing the genotoxic response characteristics of similar rodent and human tissues we can determine for particular classes of chemicals and particular types of genetic events (of the type associated with malignancy) the species sensitivity constants. These interspecies sensitivity constants will provide information directly applicable to the risk assessment process.

Project Length and Cost: 1 yr FY89: $75K

Project Officer: M. Moore (OHR/HERL-RTP) (919) 541-3933

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.2 Inter/Intraspecies Extrapolation: Interspecies Sensitivities

Status: Ongoing

Title: Dosimetric Analysis of Chemicals in Human and Rodent Tissue Response Models

Description: To determine the dosimetric relationships between exposure, dose to the DNA of target tissue, and genotoxic effects. These relationships will be used to define the use of DNA adducts as internal target dose measures. The use of DNA adducts as target dose may more accurately define specific dose response relationships with respect to dose and will be useful in future species-species and route-route extrapolations.

To determine the dosimetric relationships between exposure and dose to the target and genetic effects, this research effort will expose rodents, isolate peripheral lymphocytes, and determine both sister-chromatid-exchange frequencies, a measure of genetic damage, and DNA adducts, a measure of exposure dose. Preliminary studies will commence with animals exposed by gavage or I.P. in order to clarify the parameters or treatment time, DNA adduct persistence and repair, and structural identification of the individual adducts. The chemical selected for study is Diazaquone (AZQ), a cancer therapeutic agent. A study population has been identified who are currently being treated with AZQ. Blood samples will be obtained from these patients and SCE and DNA adducts quantitated. Similar studies will be performed using exposed rodents. DNA adducts will be identified by use of organic synthetic techniques. DNA adduct/SCE response curves will be generated from humans and rodents exposed in vitro and in vivo. The data will be fitted to the parallelogram model and extrapolation constants derived.

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. Although this project is centered on genetic effects, the development of exposure-dose relationships using blood lymphocytes is also applicable to other toxicological endpoints where systemic exposure is a requirement for the induction of adverse health effects. The product of this research will be an improved method for species-species extrapolation in risk assessment.

Identified Results: DNA-adduct-response potency parameters for AZQ exposed rodents: 7/91 (report and journal article)

DNA adduct response potency parameters for exposed humans and derivation of interspecies sensitivity constants: 8/92 (report and journal article)

Usefulness of Results: The experimental derivation of interspecies (rodent-human) sensitivity constants for specific classes of chemicals will be a major step in reducing the uncertainties in cancer risk assessment. This uncertainty is due to the assumption that man is equal to the most sensitive rodent species in terms of sensitivity to chemical carcinogens. The results of this project on AZQ-DNA adduct dosimetry will feed into an existing program on interspecies sensitivity using AZQ as an agent to which exposed tissues can be obtained from rodents and humans.

Project Length and Cost: 3 yr FY89: $290K

Project Officer: S. Nesnow (OHR/HERL-RTP) (919) 541-3847

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.2 Inter/Intraspecies Extrapolation: Interspecies Sensitivities

Status: New

Title: Dose-Response Relationships at Low Carcinogen Concentrations in a Small Fish Model

Description: This study is designed to further evaluate the small fish model, using the Japanese Medaka, for its utility as a carcinogenesis bioassay system. The primary objectives of this effort are 1) to determine bioassay parameters to measure a carcinogenic incidence below the 1% level, 2) to describe the dose-response curve for diethylnitrosamine-induced hepatic neoplastic lesions in this system, and 3) to further validate Medaka as a model for human carcinogenic potential.

The study will consist of preliminary experiments to, among other things, determine appropriate age and exposure parameters for maximizing response, characterize pharmacokinetics and dosimetry, evaluate progression of DEN lesions, standardize histopathological examination, and determine the statistical validity of a proposed design for the definitive bioassay.

The study will be carried out by investigators at the Gulf Coast Research Laboratory under a Cooperative Agreement with that institution. Principal investigators for this

project are experienced in large-scale experiments with this model system. The project will be jointly funded from both Federal and industrial sources. Currently, funding for the project from the Federal side is available through Interagency Agreements with the Army and Air Force in addition to RIHRA funds. A project oversight group made up of representatives from funding partners has been established to interact with the investigators at GCRL.

Identified Results: This project will be important in the overall effort to validate the small fish model carcinogenesis bioassay. More specifically, the project is expected to contribute to our understanding of DEN carcinogenesis as a surrogate for other genotoxic carcinogens and is expected to provide data on dose response at incidence levels of 1% or less.

Usefulness of Results: The results of these studies will have direct benefit for ongoing fish work at ERL-DULUTH. In addition, a statistically significant dose-response curve for carcinogenicity at low levels will be useful in evaluating current approaches to low-dose extrapolation for all cancer risk assessments.

Project Length and Cost: FY89: $60K

Project Officer: W. Farland (OHEA/CAG) (202) 382-7315

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Topic: Biologically Based Dose-Response Models

Issue: 4.1.2 Inter/Intraspecies Extrapolation: Interspecies Sensitivities

Status: New

Title: Interspecies Extrapolation for Oncogene Activation and Adduct Formation

Description: This effort will use specimens of human bronchus from surgical or autopsy materials and rodent lungs, specifically Strain A/J mice, as the experimental tissues for extrapolation and comparison experiments. Tissues will be exposed to five different environmental suspect human carcinogens, either in vivo (for Strain A/J mice) or in explant culture (human bronchus and mouse lung tissues) and will remain in culture until the development of preneoplastic lesions is observed. The lesions will be quantitated and then excised, or some will be removed for histopathologic analysis. Lesions that have been excised will be subjected to nucleic acid extraction, and the DNA amplified at least 100,000 fold by the polymerase chain reaction (PCR) to yield enough DNA for direct sequencing by the Sanger dideoxy technique. Alterations in the sequence of the ras family of oncogenes (H,K, and N-ras) will be examined. The identity and quantity of DNA adducts will be determined and their relationship to DNA sequence changes in the ras oncogene family will be investigated.

Identified Results:

1. Identification of adducts produced by human carcinogens in Strain A/J mouse lungs (journal article) 9/91

2. Comparison between the adduct profile produced by several human carconogens in human bronchus and Strain A/J mice (journal article) 5/92

3. Ras oncogene mutations in human lung lesions and Strain A/J mice and the relationship to adduct formation (journal article) 9/92

Usefulness of Results: This project is designed to determine if changes at the DNA sequence level induced by relevant human carcinogens are similar in humans and rodents. Therefore, this project concerns species-to-species extrapolation of the effects of carcinogens in an important human cancer target tissue: the lung. At present, we do not know how early in the cancer process that ras oncogene alterations take place, or if the temporal activation pattern is similar in rodents and humans. Insight into these questions will lead to determination of whether the mechanism of action of carcinogens is similar in humans and rodents, and this evaluation will undoubtedly bear on judgments as to what kinds of risk estimation models are appropriate for these human carcinogens.

Project Length and Cost: 2 yr FY90: $135K

Project Officer: M. Mass (OHR/HERL-RTP) (919) 541-3514

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Topic: Biologically Based Dose-Response Models

Issue: 4.3 Mechanistic Variation

Status: New

Title: Derivation of the Parameters of the Moolgavkar-Knudson Two-State Carcinogenesis Model Using Carcinogens and Tumor Promoters

Description: This project is designed to provide information primarily on the effect of tumor promoters and nongenotoxic carcinogens on the development of stages in the carcinogenesis process. Normal and premalignant cell populations and their kinetics of replication will be quantitatively analyzed.

Derivation of the parameters of the Moolgavkar-Knudson model requires experimental systems in which the number of normal target cells are known, the number of intermediate cells are recognized, the transition rates are observable, the states of terminal differentiation and proliferation can be recognized and numbers quantitated, and the malignant lesion can be scored. Experimental models where recognition of these stages of cells is possible include rat liver focus induction, rat mammary tumor models, mouse skin models in vivo or in vitro, or rat tracheal cells in vivo or in vitro. Tissues chosen from the appropriate experimental model will be exposed to carcinogens to generate the initiated "intermediate cell," and the transition rate constant (mu-1) will be calculated from appearance of markers of the initiated phenotype known for each tissue. The constants relating terminal cell differentiation and proliferation (alpha and beta), which will give information on the effect of tumor promoters, might be calculated by classical autoradiography. The transition rate constant between intermediate cells and malignant cells (mu-2) can be quantitated biologically. Data will be examined for appropriateness of fit to the Moolgavkar-Knudson model, a modified model will be constructed or other models will be examined, and the relevant constants extracted.

Experimental and epidemiologic studies have provided strong evidence that carcinogenesis is a process occurring in more than one stage. The Moolgavkar-Knudson model predicts that the first stage, initiation, is a mutation involving transition of a normal cell to an "intermediate cell" with a defect in control of cell proliferation. The numbers and proportions of these cells within the normal population are dependent upon the intermediate cell's program to terminally differentiate and die, or to remain proliferative; both these activities (induction of terminal differentiation and proliferative activation) are known characteristic actions of tumor promoters and antipromoters. This implies that the number of intermediate (initiated) cells can be regulated by tumor promoters. A second mutation is postulated to be responsible for the intermediate cell's conversion to a malignant cell that is strictly committed to proliferation. Therefore, cancer can be envisioned as a process that can have genetic components (creation of intermediate and malignant cells by mutation), and epigenetic components such as regulation of the number of offspring of intermediate cells by tumor promoters. The formal relationship has been defined by the Moolgavkar-Knudson incidence equation, which uses constants to define the probabilities of intermediate cell and malignant cell creation, and rates of proliferation or differentiation.

Identified Results: Determination of the transition rate constant for generation of intermediate cells for several carcinogens: 8/91 (report and journal article)

Determination of the effect of tumor promoters on constants alpha and beta: 8/92 (report and journal article)

The Moolgavkar-Knudson model for assessing the biological effects of carcinogens and tumor promoters: Application to several data sets and implications to risk assessment: 8/93 (report and journal article)

Usefulness of Results: This research effort has direct application to the RIHRA process in that the Moolgavkar-Knudson model provides a means by which to quantitate the effects of carcinogens and tumor promoters, and their postulated differential effects on initiated cells. The Agency has made regulatory decisions involving agents such as gasoline vapors, trichloroethylene, and dioxin whose mechanism of action as carcinogens do not fall into the category of genotoxic; therefore, it is necessary that the Agency develop proper guidelines based upon the mechanism of action of these compounds that will ensure more appropriate risk estimations. The project has direct benefit for the Agency's need to develop relevant risk assessments. Genotoxic carcinogens, which are present in all environmental media over which the Agency has purview, appear to be significant human health hazards, and our understanding of their mechanism of action is poor.

Project Length and Cost: 3 yr FY89: $170K

Project Officer: M. Mass (OHR/HERL-RTP) (919) 541-3514

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Topic: Biologically Based Dose-Response Models

Issue: 4.3 Mechanistic Variation

Status: New

Title: Comparative Studies on Changes in Gene Expression During Carcinogenesis in Human and Rodent Cells after Exposure to Nongenotoxic Carcinogens

Description: This project will examine through molecular techniques the changes in gene expression occurring in both human and rodent cells exposed to nongenotoxic carcinogens. The purpose of the effort is to determine if similar mechanisms are responsible for the cancer process in the two species.

The mechanisms by which nongenotoxic carcinogens produce carcinogenic effects are not fully known in either rodent or human tissues. Many diverse mechanisms have been proposed. Although the initial mechanisms of action often differ with respect to the initiation of cancer, some commonalities may exist in the stages of the carcinogenic process after the initial chemical insult. It is these stages that need to be measured by state-of-the-art molecular biological techniques, especially at the level of gene transcription and gene expression. Possible endpoints include 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.

Primary mesenchymal or epithelial cells of rodent and human origin will be cultured and exposed to nongenotoxic carcinogens. The effects of exposure will be measured at the molecular level in terms of alterations in gene expression of those genes thought to be associated with the induction or progression of the cancer process. Histological, morphological, and enzymatic changes will be examined to correlate with the changes in gene expression markers, particularly with respect to preneoplastic or neoplastic changes. If carcinogenesis assays are not available for a specific human cell type, such assays could be developed by use of gene transfer techniques.

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. Nongenotoxic carcinogens are more difficult to study from a mechanistic point of view due to the initial diversity of potential mechanisms. By investigating and measuring the events subsequent to the initiation of the cancer process, such as the changes in gene transcription and gene expression that occur in the cell leading to tumorigenesis, this research effort may uncover a more unifying mechanism that would simplify the assessment of these chemicals as well as make it easier to explain differences between human and rodent responses.

Identified Results: Determination of the changes in gene expression in human cells after exposure to select nongenotoxic carcinogens (peroxisome proliferators, hormones): 8/91 (report and journal article)

Determination of the changes in gene expression in rodent cell after exposure to select nongenotoxic carcinogens (peroxisome proliferators, hormones): 10/91 (report and journal article)

Determination of the changes in gene expression in human cells after exposure to select nongenotoxic carcinogens (tumor promoters, hyperplasiogens): 7/92 (report and journal)

Determination of the changes in gene expression in rodent cell after exposure to select nongenotoxic carcinogens (tumor promoters, hyperplasiogens): 9/92 (report and journal article)

Usefulness of Results: In the short term, the judicious selection of chemicals to study based on chemicals requiring or soon to require regulatory action, will provide extremely important information needed for the risk assessment process of those chemicals. The long-term use of these products could be the simplification of the risk assessment process of nongenotoxic carcinogens through a unifying mechanistic hypothesis and through a more facile extrapolation of data from cancer studies in rodents to the human situation.

Project Length and Cost: 3 yr FY89: $120K

Project Officer: S. Nesnow (OHR/HERL-RTP) (919) 541-3847

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Topic: Biologically Based Dose-Response Models

Issue: 4.1 Inter/Intraspecies Extrapolation

Title: Molecular Markers as Indicators of Chemically Induced Altered Foci

Description: The principal objectives of this research are to evaluate the following factors in mouse liver and rat liver and kidney following treatment with non-genotoxic carcinogens:

1. Ability of tumor markers to consistently detect altered foci, nodules and carcinomas from mouse and rat tissues
2. Growth rates and time-to-appearance of nodules and carcinomas
3. Frequency of phenotypic complexity as number of markers expressed/ normal tissue, altered foci, nodules or carcinomas per unit time
4. Involvement of normal and activated p21 ras in the carcinogenic process.

The project will be initiated to complement the Biochemical and Molecular Toxicology Branch's studies into the carcinogenicity of drinking water chemicals with information on the molecular mechanisms underlying the nongenotoxic carcinogenic process. Research will focus on the ability of tumor markers to consistently detect altered foci, nodules and carcinomas and measurement of growth rates and time-to-appearance of nodules and carcinomas. Six enzyme-altered foci will be studied as potential markers for hepatocarcinomas in the B6C3F1 mouse and the F-344 rat:

1. p21 ras (both normal and activated)
2. p110 myc
3. Tumor-associated isozyme of aldehyde dehydrogenase (BALDH)
4. Glutathione-S-transferase (GST-P)
5. Gammaglutamyl transpeptidase (GGTase)
6. Glucose-6-Phosphatase (G-6-P)

Previous research on a large number of samples has yielded valuable insights for future research directions. The studies have progressed to the point where reliable results have been obtained with several immunohistochemical markers. Continued studies on livers from animals exposed to chlorinated acetic acids or phthalate esters are expected to more

clearly define the markers most useful for quantitation and interpretation of the carcinogenesis process.

Future research will involve more emphasis on different markers and use of autoradiography to estimate growth rates of foci and nodules. These estimates are considered worthwhile because previous research indicated some markers are much more useful than others, and will contribute to development of mathematical models in carcinogenesis.

Identified Results:

1. Establish molecular marker profiles for preneoplastic foci, nodules and carcinomas in mouse liver (12/90) and rat liver (8/91) and kidney (6/92): (reports and journal articles)

2. Determine growth rates of altered foci, nodules and carcinomas in mouse liver (2/91) and rat liver (12/91) and kidney (9/92): (reports and journal articles)

3. Biologically based models for assessing effects on dichloroacetic acid and phthalate esters in mice (2/91) and rats (12/91): (reports and journal articles)

Usefulness of Results: Data from the proposed studies should contribute to an understanding of non-genotoxic carcinogenesis mechanisms, development of mathematical models of carcinogenesis and assist the Agency in extrapolating test results to low environmental levels. These data can be used to test mathematical computer models using the biologically based two-stage carcinogenesis model (Moolgavkar model). This model is attractive for risk assessment because it is based on, and constrained by, biological events at the cellular level. Immunohistochemical techniques appear especially well suited to mathematical modelling because they permit clear identification of putative preneoplastic lesions from normal background tissue to facilitate quantitative size determination by computer-assisted image analysis. Understanding the mechanisms of action and development of mathematical models of carcinogenesis may permit more accurate prediction of risks associated with environmental exposure to chemicals based on animal studies.

Project Length and Cost: 3 yr FY90: $90K

Project Officer: B. Daniel (OHR/HERL-Cin) (513) 576-7401

D.5 CROSS-CUTTING ISSUES

In developing the research focuses under Topic 4, several activities were identified that would benefit the risk assessment process across target systems (processes). The genesis of much of this research lies in ECAO-Cincinnati, where those scientists have had the lead in implementing the RfD process. This effort has included the identification of critical data gaps, the resolution of which would improve quantitative risk assessment in general and specifically the RfD methodology. Recognition of these research needs is reflected in support (OHEA/RURA) for the continuation of efforts to improve the statistical basis for the derivation of the RfD and the development of protocols to better estimate health risks associated with short-term exposures. Additional, proposed projects to complement and expand those activities would address the characterization and quantification of severity of toxic responses and the further development of models that incorporate varying exposure durations into the risk estimation.

Two other "generic" projects have been proposed. One is directed at convening workshops to address specific issues that are identified in the development and implementation of the RIHRA program. The second project attempts to benefit from

the extensive regulatory databases accumulated within the Agency (e.g., in OPP). Research efforts would entail organization of those data for subsequent evaluation on a variety of issues, including pharmacokinetics, species comparability, SAR relationships, and the relationship of various toxicities over varying dose and exposure conditions. (To implement this "database" project would require substantial resources and involvement of a number of Program Offices. As such, funding is not recommended under the RIHRA program. However, it is important to present this project in light of its substantial impact if a coordinated Agency effort could be funded.)

Most of the environmental pollutants considered by EPA for regulation are noncarcinogens, i.e., the major toxic effect is something other than cancer. The risk assessment procedures for noncarcinogens, however, lag behind those for carcinogens in terms of quantitative development. In 1980, EPA published the Guidelines for Ambient Water Quality Criteria, which outlined risk assessment procedures for both carcinogenic and general noncarcinogenic health effects. Since 1980, only minor changes have been implemented for noncarcinogens. For carcinogens, EPA generates a dose-risk relationship that can be applied to any exposure level. In contrast, for noncarcinogens, EPA generates a Reference Dose (RfD). Exposures corresponding to intakes below the RfD are presumed to be without appreciable risk, while those above the RfD are presumed to be linked to some unknown risk. One reason for this difference in procedures is the difference in complexity of the corresponding toxicity data. Cancer data usually include only one type of effect—malignant tumors—which are usually considered fatal. In contrast, noncancer toxicity commonly involves several types of effects, in different organs, and of differing levels of severity. Modeling the multivariate noncancer response constrained by the presumed existence of a theoretical threshold is much more difficult than modeling cancer risk.

One weakness inherent in current procedures is that duration of exposure is not explicitly factored into the risk estimation. For example, the calculation of the RfD only involves one selected data point from the entire set of toxicity data. One recent EPA effort to clarify and improve the noncancer risk assessment is the preparation of Agency-consensus Risk Assessment Guidelines for Noncarcinogenic Health Effects. In these Guidelines and in recent OHEA publications, models are discussed that allow characterization of health risk over a range of dose levels and exposure durations. Research on these models will substantially change the procedures for noncancer risk estimation, improving both the accuracy and breadth of application of the risk estimates.

Both qualitative and quantitative guidance is needed to define adverse health effects and to characterize their biological significance (i.e., severity). Accordingly, the severity project involves both biological and statistical issues. Some of the research topics include the relationship between subtle effects from short-term exposure and more severe effects that follow long-term exposure; mechanistic relationships; statistical and graphical methods for evaluating multivariate relationships (e.g., dose and duration predicting multiple effects).

The development of the noncancer guidelines has been greatly facilitated by several workshops and symposia sponsored during the past five years by ECAO-Cincinnati. In the numerous papers and recommendations from these meetings, the dominant quantitative issue is the involvement of more, if not all, of the toxicity data in the risk estimation procedure. The research tasks described herein address some of those recommendations, focusing on the characterization of toxic severity, on the inclusion of exposure duration as a model covariate, and on the calculation of probabilistic risk of unspecified toxic effects.

The OHEA/RURA base program on generic issues in FY89 includes specific projects that represent extensions of work initiated in FY88 on probabilistic characterization of uncertainty factors, use of dose-response models for estimating RfDs, severity/adversity characterization efforts, and use of less-than-lifetime exposure data in risk assessment. The base program for FY89 will provide explicit examples of alternatives to the current RfD procedure. The severity projects proposed herein will also demonstrate alternatives and improvements to the current procedure. When both sets of projects (RURA base and proposed RIHRA) are completed in FY90 or FY91, the results should be combined into a collection of proposed revisions to the Guidelines for Noncarcinogenic Health Effects (in draft, planned completion, FY90).

Topic: Workshops on Identified Issues in RIHRA Research Program

Status: New Project Funded Under FY89 OHEA/RURA

Description: This activity is designed to convene workshops of experts to address specific issues identified in the development and conduct of the RIHRA program. The topics will often represent areas for which research strategies need to be developed that identify and prioritize the most important questions. Research efforts can then be directed to answer these questions. NAS and ILSI are currently designated as workshop coordinators.

Identified Results: Each workshop will produce a strategy document that will be utilized by the RIHRA Subcommittee in research planning (and resource allocations).

Usefulness of Results: The major benefactor of these workshops will be the RIHRA program and, in turn, Agency risk assessors. For several areas of uncertainty, there is no existing consensus formula as to the best approach. These workshops will serve to clarify these issues for a given topic and produce a research strategy that represents the most current scientific knowledge. Such guidance will reduce the probability of embarking on haphazard, less credible research projects. The end result will be the efficient use of RIHRA resources and the production of a quality product that will further the risk assessment process.

Project Length and Cost: Continuous FY89: $150K; RIHRA Committee will set out-year amounts

Project Officer: W. Farland (OHEA) (202) 382-7315

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.2 Exposure Scenarios: Sensitivity of Endpoints as a Function of Dose

Status: New RIHRA project

Title: Severity of Toxic Response Stratification and Modeling Project

Description: Essential to the use of severity data in risk assessment is the identification of scientifically credible criteria to define an effect as adverse, and to establish the relative severity of each effect. The range of potential toxicological effects may be construed as of uncertain significance,adverse, compensatory or adaptive. This judgement will vary as a function not only of the observed effects, but perhaps most importantly, the target organ ortarget system involved. In addition, for purposes of hazard assessment severitystratification within the adverse effects would be of significant utility. The toxicology of the various organ systems (e.g. hematopoietic, immunologic, and nervous) need to be reviewed in the context of establishing criteria that constitute or define an adverse effects of these organ systems. A fundamental aspect of this effort is to establish on an organ-specific basis the relationship between histopathological progression, functional impairment of the organ system,and ultimately, the organism. This effort will build upon ongoing activity in the area of systemic toxicity to define both qualitative and quantitative guidelines to stratify the spectrum of critical effects in terms of their degree of adversity. The general approach will be to analyze the existing literature for mechanistic theories or statistical models, and to consult experts in the appropriate fields to develop guidelines for the

interpretation and modeling of effects with respect to severity. This research effort will lead to a practical, qualitative ranking scheme that allows for systematic determination of equivalent severity scores for the spectrum of effects likely to be reported in the literature, as well as statistical models that can be used to describe a dose-severity relationship for use in risk assessment. Reversible or transient effects and "u-shaped" dose-response curves, which are often reported but generally dismissed as not significant, will also receive special attention. Some chemicals, such as some metals, are essential elements and consistently display such a curve. The u-shaped curves are particularly difficult to evaluate and model. The peculiar influence of threshold parameters on model behavior is not will understood and needs to be investigated on both statistical and numerical grounds, since many such models must often be constrained at the extremes of the dose-response relationship.

Identified Results:

1. Identification of standard criteria to define adverse effects
2. Development of Agency-wide guidance for qualitatively stratifying endpoints and integrate the relationship between histopathological progression, organ dysfunction and organismal disability
3. Development of an overall severity ranking scheme and documentation for major organ toxicity
4. Development and improvement of statistical and graphical methods for evaluating multivariate relationships
5. Development of the following monographs:
   a) correlation of in vitro and in vivo immunotoxicity
   b) significance of reversible/transient effects
   c) mechanistic explanations for u-shaped dose-response curves for essential and nonessential chemicals
   d) numerical stability and robustness of categorical regression models with and without threshold parameters
   e) concordance of toxicological endpoints among species

Usefulness of Results: Applications of the results of this research are both qualitative and quantitative. A potential quantitative application arises directly from the foregoing in that no allowance is made for adjusting the RfDf or severity of effect, except when the RfD is based on a LOAEL. In the latter case, limited guidance as to the magnitude of the adjustment is given. The issue is significant because of the wide range of severity of first-observed effects among toxicological studies. A biologically based severity ranking scheme would provide a basis for rational adjustment of the RfD on this basis. In addition, for essential chemicals, guidance can be given to ensure that the RfD does not drop below the essential intake level. The primary product of this research effort, the ranking scheme, is also essential for the development and implementation of dose-risk models. The RfD does not provide any indication of the health risk for higher doses. In contrast, many regulatory efforts in EPA evaluate health risks at existing rather than target, exposure levels and could therefore benefit from the availability of such risk models.

Project Length and Cost: 3 yr FY89: $140K

Project Officers: Richard Hertzberg, Deborah McKean (OHEA/ECAO-Cin) (513) 569-7531
M. Davis (OHEA/ECAO-RTP) (919) 541-4162

Topic: Biologically Based Dose-Response Models

Issue: 4.2.2 Exposure Scenarios: Sensitivity of Endpoints as a Function of Dose

Status: Ongoing OHEA/RURA project

Title: Research to Support Use of Short-Term, Episodic and Other Less-Than-Lifetime Exposure Data in Risk Assessment

Description: The evaluation of risk from exposure to toxic substances forsome fraction of a human lifetime is the subject of ongoing research by the EPA's ECAO. Reference Doses (RfDs) have been used for subchronic and chronic exposures and Health Advisories have been used to address risks from 1-Day, 10-Day andLonger Term exposures in drinking water. Both methods require adequate toxicological data for the derivation of health-based numbers. Reportable Quantities for Acute Mammalian Toxicity address acute exposures and result in a number useful for priority setting. The RQ is of limited use to risk assessors because it does not project "safe" and "unsafe" doses. In the absence of data for the exposure duration of concern, it is often necessary to project theoretical thresholds from limited data sets. A more useful method would be to generate estimated dose-response curves from limited data. Toward these ends, a number of studies have examined the relationship between measures of acute toxicity (LD50, ED50, etc.) and experimentally determined NOAELs. As proposed, this "ratios approach" would be used to estimatea NOAEL for a chemical from limited data. It is not anticipated that this method be used in lieu of current RfD methodology. The probit-slope of the dose-response curves for a variety of compounds has also been examined for a variety of compounds in order to determine the feasibility of estimating the dose-response curve for a variety of endpoints based upon limited data. Both approaches include the creation of a computerized toxicity data base by accessin other existing data bases. At the opposite end of the spectrum are chemicals with a very large toxicity data base. Two procedures are being developed to facilitate visual interpretation of complex and diverse data sets. Graphics for plotting severity of effects data for dissimilar experiments on a common, human-equivalent scale f dose and duration have been used to identify combinations of dose and duration which may result in adverse effects. Three dimensional (3-D) graphics are also being developed for use when data sets are very large and complex. Statistical techniques are also being explored for generating confidence intervals around a 3-D dose-response surface. Research is also being conducted on a modification of existing nonparametric generalized rank methods for examining the effect of exposures across multiple organ systems in which the degree of severity cannot be quantified. Another project examines the relationship between temporal patterns of exposure and the severity of effects. The problem stems from the fact that the observed effects are related not only to the total dose but also to the dosing scenario.

Identified Results:

1. For each project, a computer program will be generated along with guidance for its use and application to risk assessment. Monographs on the following topics:
2. A ratios approach to estimation of NOAELs based upon acute toxicity data
3. Estimation of the probit slope of the dose-response curve based upon acute toxicity data
4. 3-D graphics for trend analysis in risk assessment
5. Graphic analysis of dissimilar data in risk assessment confidence intervals for toxicological data arrayed in three dimensions

6. Novel statistical procedure for quantitative risk analysis at multiple toxicological endpoints

7. The relationship between temporal dosing patterns and duration and the severity of observed effects.

Usefulness of Results: Specific data gaps and uses for products are described with each project description above. All of these projects will contribute to improving health risk assessment to a substantial degree. For each project, proposed guidance is expected to be developed as a result of the peer review process. In general, the methods developed will permit estimates of NOAELs and dose-response curves based upon limited data; facilitate trend analysis, response prediction, and significance testing of large data sets and data from dissimilar experiments; and provide guidance for both investigators and risk assessors on the chemical-class-specific effects of dosing patterns and duration of exposure.

Project Length and Cost: 3 yr FY89: $128K

Project Officers: C. Cubbison, C. DeRosa (OHEA/ECAO-Cin) (513) 569-7531

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Topic: Biologically Based Dose-Response Models

Issue: Cross-Issue/Cross-Target

Status: Ongoing OHEA/RURA project

Title: Reference Dose: Investigating Underlying Probability

Description: The objectives of this project are to determine probability density functions of uncertainty factors (UF's) and the statistical variablility of the NOAEL, and to test various theoretical dose response models that may improve the current Reference Dose (RfD) methodology. The approach to the former goal is to collect and analyze data from toxicology studies (at the dose group level) in order to determine the experimental variability underlying the defined uncertainty factors. Dose response models will be investigated as a means to expressing the uncertainty in the NOAEL, or replacing the NOAEL with some other estimate. Models (or procedures) to be tested currently include Crump's benchmark approach, Brown's exact NOAEL procedure, and Hertzberg's categorical regression model.

Identified Results: Several manuscripts discussing specific areas of uncertainty and dose-response modeling (4 papers completed FY89, 4 papers in preparation FY90); computerized data base of experimental studies for estimation of various uncertainties.

Usefulness of Results: The probabilistic limits on the RfD by analysis of its subcomponents and/or the calculation of more mathematically precise RfDs by way of modeling are particularly important goals for improving the credibility of quantitative risk assessment. The ability to express the RfD in a statistical framework would greatly facilitate the interpretation and use of this estimate in risk management decisions.

Project Length and Cost: 2 yr FY89: $110K

Project Officer: J. Swartout (OHEA/ECAO-Cin) (513) 546-7811

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Topic: Biologically Based Dose-Response Models

Issue: 4.2.2 Exposure Scenarios: Sensitivity of Endpoints as a Function of Dose

Status: New RIHRA project

Title: Refining Risk Assessment Models by Investigating Critical Issues in Interspecies Extrapolation

Description: This project is designed to refine risk assessment models by comparative analyses of existing clinical, epidemiologic, and toxicology data concerning the effects of well-characterized chemical exposures. Extensive animal and human data are typically generated in the process of development and evaluation of new pharmaceutical agents and other consumer products. These data, the product of many millions of dollars of research, may include short-term and long-term mortality and carcinogenesis testing in experimental animals, organ system toxicity, dose-limiting toxicity, as well as extensive pharmacokinetic and other biologic data in both animals and man, and epidemiologic data concerning individual differences and long-term effects in humans. Because the focus of the use of the toxicologic data tends to be the development of clinical trials, and the results of the clinical trials ultimately speak for themselves, rigorous comparisons of the toxicologic and clinical findings have rarely been undertaken. For many classes of agents, even relatively simple associations, such as whether the dose-limiting organ toxicity was similar in animals and humans, have not been well described. Where the findings in human and toxicologic systems coincide, they reinforce the utility of the toxicologic models; where differences are observed, they indicate critical needs for further refining the toxicologic models. While most effort will use existing data, focusing on pharmacoepidemiologic data, existing data may be supplemented by attempts at filling critical data needs under this project and by the development of protocols for filling larger data gaps in other studies. We plan to pursue several aspects of these investigations through collaborations with federal agencies such as the FDA and NCI or other sources possessing the required data.

Identified Results: Interspecies comparisons of effects of well-characterized exposures in animal systems and in humans.

Usefulness of Results: An understanding of the concordance or discordance between well-defined exposure/effect links in humans with exposure/effect links predicted by experimental systems widely used for risk assessment should provide vital data for validating and refining approaches used for risk assessment.

Project Length and Cost: 3 yr FY89: $80K

Project Officer: R. Everson (OHR/HERL-RTP) (919) 541-1963

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