American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005

Dale

memo

Date: January 25, 1993

From: Mary Paxton

To: Benzene Task Force

Re: Update

The next meeting of the Benzene Task Force is scheduled for Tuesday, March 2, at API's Washington headquarters in Room 907. Please plan to be here by 9 a.m. for a full day's meeting. An agenda will follow. Please notify Roxie Landry (202-682-8330) by February 24 of whether you plan to attend or not.

Attached are copies of the proposals submitted by Lovelace on benzene metabolism and dosimetry and on biomarkers of benzene exposure, which we will decide about at the March meeting.

Draft Proposal

for the Study of

BIOMARKERS OF EXPOSURE TO BENZENE

Submitted to the

American Petroleum Institute

by the

Inhalation Toxicology Research Institute

P. O. Box 5890

Albuquerque, NM 87185

Operated by the

Lovelace Biomedical and Environmental Research Institute

for the

U. S. Department of Energy

under Contract No. DE-AC04-76EV01013

November 1992

ITRI Contact:

C. H. Hobbs, DVM
ITRI Assistant Director
505-845-1045

or

R. F. Henderson, PhD
Principal Investigator
505-845-1154

I. INTRODUCTION

Benzene is a known hematotoxin and leukemogen in humans. To determine the health risks associated with benzene exposure in a quantitative fashion, it is essential to determine dose/response relationships. The dose of greatest interest is the biologically effective dose, i.e., the dose that causes a change in the biological system that leads to an adverse health effect. In the case of benzene, the mechanism by which benzene and/or its metabolites induces acute myelogenous leukemia in humans is not known. In the absence of a mechanism, one cannot determine the true biologically effective dose, but the dose to the target tissue, the bone marrow can be determined.

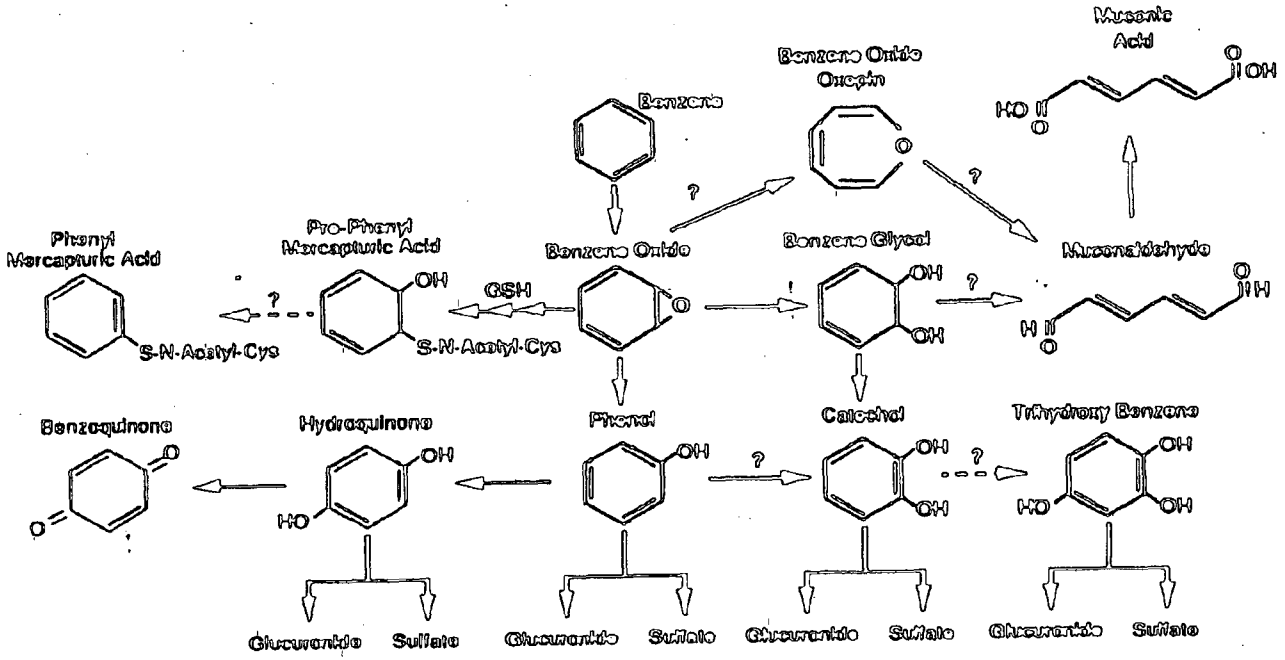
One approach to quantitating dose is through the use of biological markers of exposure, as described by the National Research Council (1987). In the past, most toxicology studies were designed to monitor only the external exposure regimen and a developing adverse health effect. Information from such studies may be deceiving, because the external dose may not be linearly related to the internal body dose or the dose to the critical site of toxicity or most importantly, to the biologically effective dose. The ideal state of knowledge would be to know the quantitative relationships between the external exposure, internal dose, dose to target tissue and biologically effective dose. If the kinetics of formation and removal of the specific markers is known, than mathematical models can be used to described the relationship between the various markers following different exposure regimens. Such a model will allow the use of readily available markers, such as those in blood or urine, to predict the level of less available markers of exposure in the target tissue, such as the bone marrow.

II. BACKGROUND

The ITRI has had extensive experience in measuring the dosimetry of benzene administered orally or by inhalation. We have determined the effect of species, route of exposure, and exposure concentration on the internal dose, and area under the curve for major metabolites in tissues of exposed rats and mice (Sabourin *et al.*, 1989). These studies indicated that the production of putative toxic metabolites (muconaldehye and hydroquinone) was favored at lower exposure concentrations. Limited studies in nonhuman primates suggested that hydroquinone production was also favored at low exposure concentrations in those species (Sabourin *et al.*, 1992). Studies to determine the effect of repeated exposures to benzene on its metabolism showed little change from the results following single exposures (Sabourin, *et al.*, 1990). Although area under the curve values were not obtained for the bone marrow, the studies at ITRI showed that bone marrow, the target tissue for benzene toxicity, contained metabolites that reflected those in the blood.

Biological markers of exposure to benzene should be benzene specific (i.e., are produced only by benzene) and quantitatively relatable to prior exposures. Based on what is known about benzene metabolism (Fig. 1), biological markers specific to benzene must be related to either benzene itself or to the benzene epoxide that is the first step in metabolism of benzene. The benzene oxide can react with glutathione to form phenyl mercapturic acids or it can act to phenylate nucleophilic sites on proteins or DNA. The benzene oxide can also be further oxidized to form the ring-opened products, such as muconaldehyde or muconic acid. Benzene oxide can also be metabolized to phenolic products, the traditional biomarker of benzene exposure, but phenol products are not benzene specific. Phenol is present in the environment and is a metabolic product of some aromatic amino acids. Urinary phenol was

FIGURE 1. METABOLIC PATHWAYS FOR BENZENE*



* Adapted from Henderson et al. 1989

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valid for exposures to 10 ppm benzene or above but, since the lowering of regulated exposures to 1 ppm, can no longer be used due to the high background levels from nonbenzene sources.

Besides being chemical specific, biological markers that are retained in the body with a long halflife are desirable because they can be used to integrate exposures over a longer time period. However, shorter halflived markers often occur in larger amounts and can be more easily detected soon after a single exposure. Good use can be made of a panel of biological markers of varying halflives. Biological markers that have a short halflife, such as a vapor in exhaled breath or metabolites in the urine, may be present in large amounts soon after the exposure, but may not be detectable at later times. Other markers, such as blood protein adducts or DNA adducts, may be formed in only trace amounts after a single exposure but are cleared more slowly and will accumulate with continued exposure. Thus one could distinguish between a recent single exposure, an ongoing exposure, and a past exposure with no recent exposures by the levels of the biological markers having different halflives (Fig. 2).

III. SPECIFIC AIMS OF THIS PROPOSAL

The purpose of the proposed work is to test the hypothesis that a panel of biomarkers of benzene exposures can be used to quantify prior exposures to benzene. This will be achieved by completing the following specific aims:

1. Develop a PBPK model describing the time course relationships between several benzene specific biological markers of various halflives. The markers will be benzene in breath and blood, phenyl mercapturic acid, phenyl guanine and muconic acid in urine, phenyl cysteine adducts in blood proteins and

FIGURE 2. POTENTIAL USE OF PANEL OF BIOMARKERS FOR ASSESSING PRIOR EXPOSURES

Marker	Marker $t_{1/2}$	Case I	Case II	Case III
A	minutes	+	+	–
B	hours	+++	+++	–
C	days	++	++	–
D	weeks	+	+++	++
E	months	+	+++	++

Case I - Recent exposure.
Case II - Ongoing exposure.
Case III - Exposures were several months ago; no recent exposure.

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hydroquinone and muconic acid in bone marrow. The latter two markers are included so that we can relate the readily available markers in breath, urine and blood to target tissue metabolites.

2. Expose mice to benzene by different exposure regimens and determine if the model allows us to distinguish between the animals that had been exposed recently, or sometime in the past or continuously.

IV. EXPERIMENTAL APPROACHES

A. Developing the Model

The experimental data that will be required for the model are the amounts of the markers produced by a given amount of benzene exposure and the rate at which the marker is removed. Much of this information is already known based on our past studies and the remainder are being developed under a separately funded project. An existing physiological model previously developed at ITRI for single exposure scenarios (Medinsky *et al.*, 1989) will be expanded to include the six biomarkers and will be modified for multiple exposure scenarios. These experimental data will be used in the development of the model describing the temporal, quantitative relationships between each of the markers and the benzene exposure levels. Exposures of mice by different regimens (see below) will be used to validate the model and determine uncertainties of the model predictions. Simusolv (Dow Chemical Co., 1990) on a microvax computer will be used. Statistical criteria provided in Simusolv will be used to test the goodness of fit and to select the best-fit pharmacokinetic model.

B. Exposure of mice by different regimens

Once the model has been developed, mice will be exposed to 1 or 10 ppm benzene once or repeatedly and the animals sacrificed either immediately after the exposures or one week or one month after the exposures. Based on our earlier work (Sabourin *et al.*, 1989), we know that either 1 or 10 ppm exposures are within the linear range for total metabolism of benzene as well as for metabolism by any of the pathways to individual metabolites. We will determine the levels of the six biomarkers in the mice following the different exposure regimens and determine if the model is valid. To determine the relationship between the readily available biomarkers under study and the less available markers of dose in the target tissue (bone marrow), we will also measure markers of exposure in the marrow. This will allow us to use the model to predict target tissue doses based on the readily available markers of exposure. If the model is not predictive of the results, we will experimentally determine the basis for the lack of validity and will correct the model.

C. Time Line

The time line for the expected completion of these studies is shown in Figure 3. Although we know that the funding will have to be renewed on a yearly basis, we have projected studies over a three year period and are assuming we will need to expose mice twice to obtain the data we need for a valid model.

V. EXPECTED RESULTS

These studies will determine if a panel of biomarkers with varying halflives can be used to characterize prior exposures to benzene in rats.

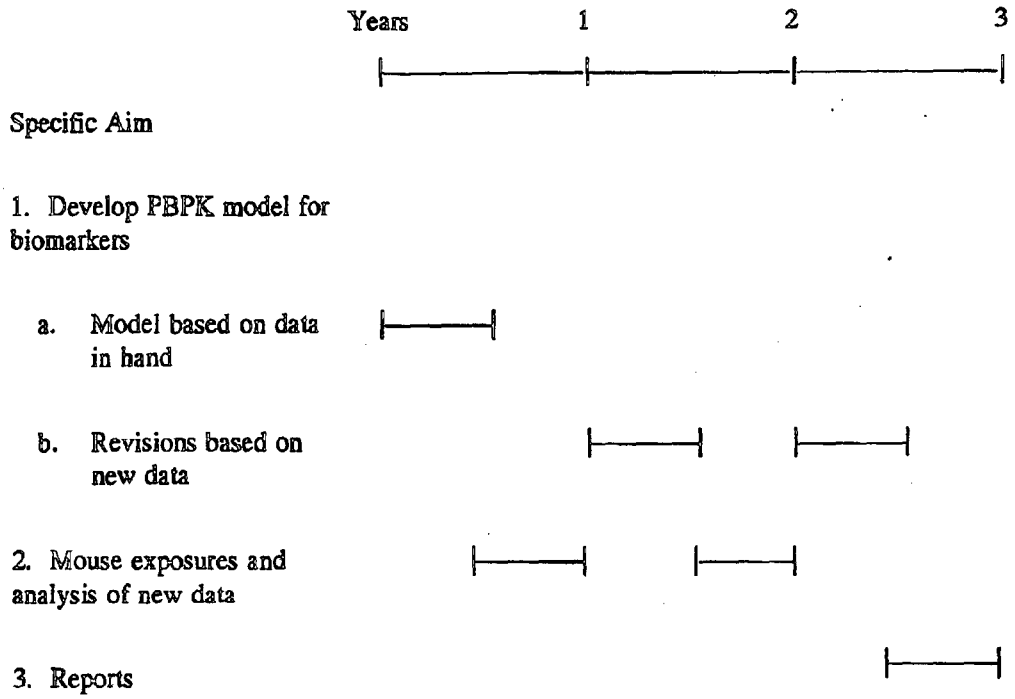


Figure 3. Time Line for Proposed Study

VI. FUTURE WORK

A logical extension of this work would be to modify the PBPK model for humans and test the validity of the human model in occupational workers with known exposures to benzene.

VII. REFERENCES

Medinsky, M. A., P. J. Sabourin, G. Lucier, L. S. Birnbaum and R. F. Henderson (1989) A physiological model for simulation of benzene metabolism by rats and mice. Toxicol. Appl. Pharmacol. 99: 193-206.

National Research Council (1987) Biological markers in environmental health research. Environ. Health Perspect. 74:3-10.

Sabourin, P. J., W. E. Bechtold, W. C. Griffith, L. S. Birnbaum, G. Lucier and R. F. Henderson (1989) Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F_1 mice. Toxicol. Appl. Pharmacol. 99:421-444.

Sabourin, P. J., J. D. Sun, J. R. MacGregor, C. M. Wehr, L. S. Birnbaum, G. W. Lucier and R. F. Henderson (1990) Effect of repeated benzene inhalation exposures on benzene metabolism, binding to hemoglobin and induction of micronuclei. Toxicol. Appl. Pharmacol. 103:452-462.

Sabourin, P. J., B. A, Muggenburg, R. C. Couch, D. Lefler, G. W. Lucier, L. S. Birnbaum and R. F. Henderson (1992) Metabolism of [14C]benzene by cynomolgus monkeys and chimpanzees. Toxicol. Appl. Pharmacol. 114:277-284.

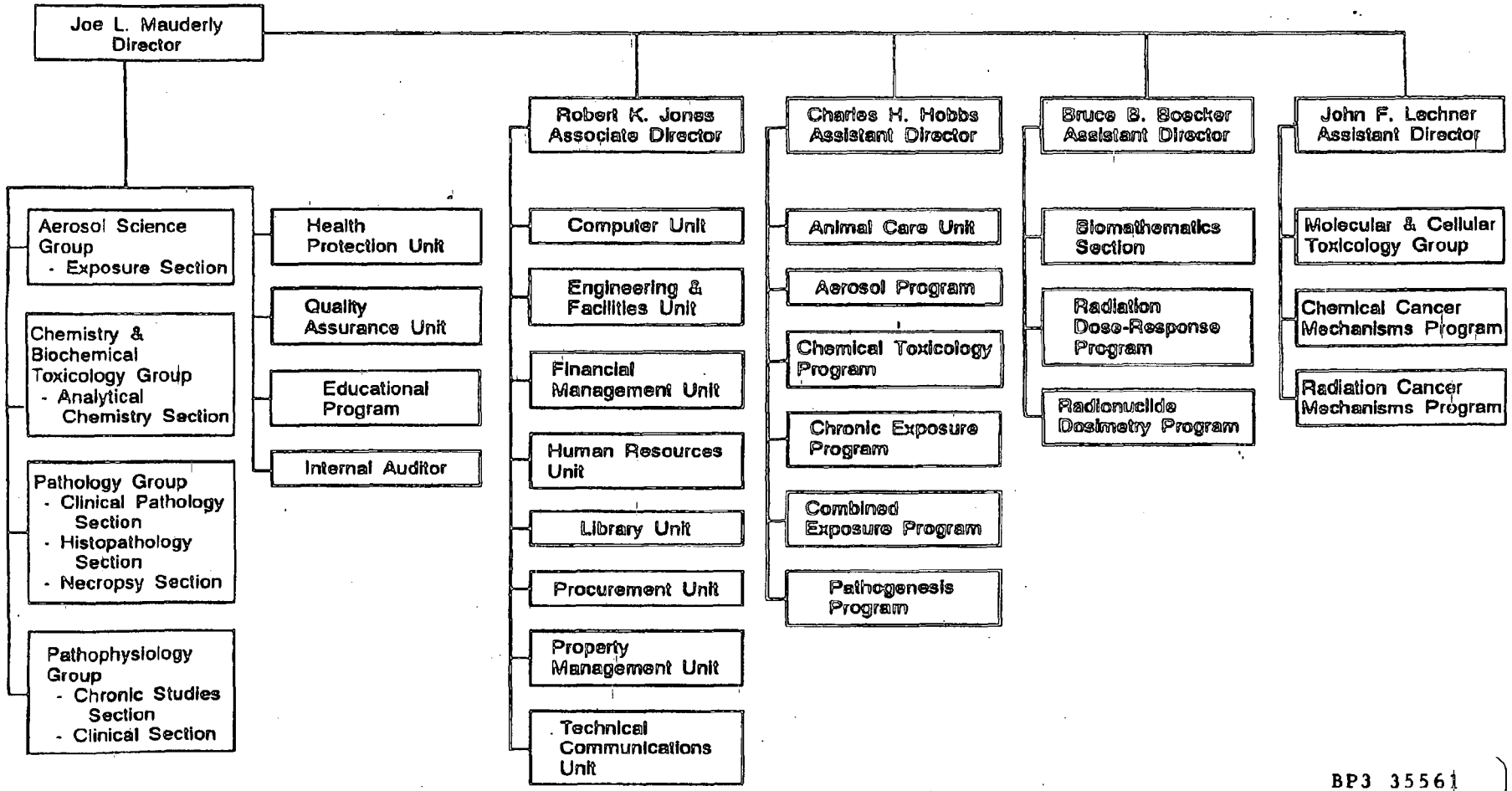
VIII. RESOURCE AND RESEARCH MANAGEMENT

The Institute's resource management structure is shown in Figure 4. ITRI's Director, Dr. Joe L. Mauderly, serves also as President of LBERI. Dr. Robert K. Jones is the Associate Director of ITRI and Vice President of LBERI. Dr. Charles H. Hobbs, Dr. John F. Lechner and Dr. Bruce B. Boecker act as Assistant Directors of ITRI. Dr. Hobbs is responsible for management of the chemical toxicology projects at ITRI, Dr. Lechner is responsible for molecular biology projects, and Dr. Boecker is in charge of projects in radiation toxicology. These five members of the Directorate provide overall institutional resource management and each has responsibility for people, space and equipment included within their specific research groups and support service units. The research project described in this proposal will be under the overall supervision of Dr. C. H. Hobbs. Each project also has a project coordinator or study director. In this case it will be Dr. R. F. Henderson.

Since the Institute is operated under contract with the DOE, we must and do comply with all federal regulations relative to personnel activities, procurement, property management, accounting standards, facility maintenance, security and health and safety procedures. We have developed and utilize a computerized management information system to account for all financial transactions and manpower data as well as for activities relating to property management and other support services activities. Monthly printed reports on current period and year-to-date transactions are provided to all appropriate personnel for management purposes. Finally, it should be noted that a year-end financial audit is conducted on the Institute's prior fiscal year financial transactions by U. S. Government auditors to assure compliance with federal regulations and to verify an appropriate indirect cost basis which is applicable to all sponsors for whom research has been conducted. In summary, we

FIGURE 4

INHALATION TOXICOLOGY RESEARCH INSTITUTE
Organizational Structure



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feel that the Institute has the effective and experienced management team required to conduct both cost-effective and high-quality research for sponsors.

IX. RESEARCH PROJECT MANAGEMENT AND PERSONNEL

A multidisciplinary team of scientists has been chosen for work on this project. Table 1 lists the members of the research team. Curriculum vitae of all professional staff are included in the Appendix.

The ITRI scientists with primary responsibility for the management and conduct of these proposed studies are Dr. C. H. Hobbs and Dr. R. F. Henderson. In the context of the Good Laboratory Practice Regulations, Dr. Henderson will be the Study Director (Principal Investigator) for the studies and as such she is responsible for overall conduct of the studies and will serve as the contact at ITRI for API for conducting the studies. Dr. Henderson is trained as a biochemical toxicologist and has extensive experience in the toxicokinetics of inhaled vapors.

Dr. William Bechtold is a chemist with experience in analyzing for benzene metabolites. He will be in charge of analyzing for benzene and its metabolites as biomarkers of benzene exposure.

Dr. I-Yinn Chang is a biomathematician who has had training in the development of PBPK models and is familiar with the benzene PBPK model developed at ITRI.

Appropriate technical and other supporting personnel will be assigned to the project to meet project objectives. The professional, technical and other supporting personnel will work as a team. Should the need arise for technical expertise not available from personnel on the team, assistance of other members of the Institute staff will be enlisted. The Institute retains

Table 1

Summary of Scientific Personnel with Substantial Involvement in the Project

Name	Degree/Discipline	Role in Study
Henderson, R. F.	Ph.D., Biochemical Toxicologist	Study Director (Principal Investigator)
Bechtold, W. E.	Ph.D., Chemist	Co-Investigator, Analysis of Metabolites
Chang, I.-Y.	M.S., Biomathematician	Co-Investigator, Development of Model

the authority to assign equivalent personnel or otherwise use Institute personnel, within the limits of the authorized budget, to best achieve the project's objectives.

X. REPORTS.

At 6-mo intervals, a letter report on the progress of the project will be submitted to API by LBERI.

The LBERI will immediately notify API of any private or governmental request for information on ongoing or completed research conducted under this Agreement, including any subpoena or other legal instrument requesting information. The Contractor will cooperate fully with any effort by API to narrow the scope of any such request, to obtain a protective order limiting the use or disclosure of information.

XI. PUBLICATIONS

API and the LBERI agree to full public disclosure of scientific information developed through this Agreement. The LBERI shall not release such information without prior API knowledge and review.

The scientific conclusions and professional judgments arising out of performance of the project will be the responsibility of the LBERI. The LBERI, however, will not publish or otherwise release data, conclusions, or manuscripts in a citable or quotable form without prior API knowledge and opportunity to comment. API opportunity to comment is for the purpose of clarification and format or editorial comments, but not for the purpose of substituting API's opinion for that of the LBERI.

XII. INSTITUTIONAL RESOURCES

A. General

The Inhalation Toxicology Research Institute (ITRI) has been operated by the Lovelace organization for the U. S. Department of Energy (DOE) or its predecessor agencies since the early 1960's. It is currently operated under a DOE five-year, cost-reimbursable, no-fee contract by the Lovelace Biomedical and Environmental Research Institute (LBERI), a subsidiary of the Lovelace Medical Foundation (LMF). LBERI's only mission is to operate ITRI for DOE and to conduct research for DOE and other sponsors where appropriate. LBERI has no financial assets other than those it is responsible for managing for the United States government or its other sponsors.

ITRI facilities are totally government owned and are located on Kirtland Air Force Base East in Albuquerque, New Mexico. These facilities contain approximately 271,000 ft^2 of space for laboratories, animal breeding and maintenance activities, offices and essential support services. Since first occupied in 1963, new additions or modifications have been added on nearly an annual basis to meet the ever-changing nature of the Institute's research program. These facilities represent one of the most modern and complete laboratories for the conduct of inhalation toxicology research that are available anywhere in the world. Pertinent details are provided below.

B. Facilities

The Institute's government-owned facilities consist of approximately 271,000 ft^2 of space. They include a central laboratory, office and small animal and primate maintenance complex, dog kennels on either side of the central laboratory and specialized facilities for machine shop, electronics shop, analytical chemistry, rodent quarantine and

rodent breeding, primate quarantine, receiving and warehouse, health protection and waste handling located more peripheral to the central complex.

From the standpoint of this proposal, the following facilities are considered to be of principal importance:

1. Modern analytical chemistry laboratories containing state-of-the-art instruments. The instrumentation includes liquid scintillation counters, spectrofluorometers, high-pressure liquid chromatographs with auto injectors and auto sample collectors, UV and VIS spectrophotometers, gas chromatograph/mass spectrometers with a computer data system, gas chromatographs, a laboratory data system for quantitation of GC and HPLC data, infrared spectrometer with a computerized data system, and a nuclear magnetic resonance spectrometer.
2. Laboratories for safely developing and testing generation procedures for inhalation exposure environments using either radiolabeled or unlabeled materials, including potential carcinogens. These laboratories are equipped with instruments for physicochemical characterization of the exposure environments.
3. Hazardous material storage and preparation area designed and constructed for storage of hazardous materials in a controlled access area. The facility has storage capabilities adjacent to a preparation area. This area provides for storage of radioactive materials in a shielded vault and storage of nonradioactive materials in a cabinet or refrigerator. The preparation room is equipped with a hot cell and three hoods for

preparing aerosol generator solutions and other operations related to preparing materials for use in inhalation exposures.

4. Inhalation exposure facilities specifically designed to allow controlled access to an area fully equipped for acute inhalation exposures.

Each of two exposure complexes within the facility has its own control room, two exposure rooms and a preparation room. Associated equipment in the facility includes an ultrasonic unit for decontamination of exposure apparatus and an electron microscope. A variety of laboratory animals have been exposed nose only in the facility, ranging in size from mice to dogs. Materials aerosolized for animal exposures have included chemical carcinogens, a variety of radioactive compounds and vapors. Exposures have included respiratory monitoring by whole-body plethysmography in some cases and the facility is equipped to make these respiratory measurements for small laboratory animals, dogs and monkeys.

5. Quarantine facilities are available for isolation and stabilization of animals received from commercial suppliers. Rigid standard operating procedures are followed during the quarantine period while the health status of newly acquired animals is evaluated.

6. A barrier-sustained, rodent housing facility is operated on a clean-dirty corridor system. All personnel must shower before entering the facility and must wear clean laboratory-supplied clothing, face mask and gloves. All materials entering the facility are sanitized (i.e., cages, lids, caps,

personnel clothing). Feed is pasteurized by suppliers and all bedding is sterilized prior to use. All items that cannot be entered through the tunnel washer or autoclaved are chemically sterilized. The animal rooms are maintained at 20 to 23°C and at 30 to 50% relative humidity under a 12 h light and dark cycle.

ITRI is a registered research facility, in compliance with Section 6 of P.L. 89-544 (as amended), the Laboratory Animal Welfare Act, and has been fully accredited by the American Association for Accreditation of Laboratory Animal Care since 1970. The last accreditation inspection was conducted in June, 1988. Animals are maintained in accordance with the recommendations in NIH Publication No. 85-23, entitled "Guide for the Care and Use of Laboratory Animals" and in compliance with the Laboratory Animal Welfare Act.

7. A cellular toxicology facility is specifically designed and staffed to test the effects of chemical toxicants, carcinogens and radiation on eukaryotic and prokaryotic cells in culture. Equally important are the capabilities in this facility to determine the *in vitro* functional capacities of cells exposed to toxicants *in vivo*. A FACSTAR PLUS (Becton-Dickinson) flow cytometer is available.

8. A comprehensive program for hazardous waste handling and disposal. Radioactive and hazardous wastes generated at the Institute are collected, treated, packaged and shipped in accordance with all EPA,

DOT and DOE regulations, and in a manner that minimizes hazard to personnel or the environment.

Hazardous chemical wastes are collected and handled in a manner consistent with the requirements of the Resource Conservation and Recovery Act (RCRA). As with radioactive wastes, all hazardous chemical wastes are shipped to EPA-approved disposal sites for final disposal.

9. In-house computer capability consisting of a network of Digital Equipment Corporation VAX series computers. It currently includes a VAX 11/780, three MicroVAX II computers and five PDP-II series computers. The latter are required for real-time data acquisition. Extensive use is also made of personal computers for automated data collection, scientific calculations, word processing and graphics. These computer resources provide facilities for collection, storage and processing of both scientific and business or management data for the total Institute.

10. Emergency electrical generation capability is provided to all essential areas of the facilities. This capability includes two diesel-powered 750 KW and two 100 KW generators. They receive routine testing on a weekly basis and require only six seconds for full activation. All of the facilities described above are incorporated into this emergency power grid.

11. A complete in-house shop capability including a machine shop, electronics shop and carpenter shop. The personnel in each shop area continue to provide the necessary experience and skills for the initial design and fabrication or subsequent modification of prototype exposure chambers, characterization devices and other instruments required at ITRI.

12. An excellent research library which provides all services required to support our research program. These include current subscription to 300 journals, over 9,500 books and approximately 19,000 documents. Local and regional interlibrary loan services are available through the University of New Mexico and a consortium of New Mexico Medical Libraries. On-line literature search services are maintained with over 100 data bases through 4 major systems. These services will be essential for providing available literature on specific chemicals which ITRI is requested to study.

Although this by no means describes the Institute's total facility and equipment resources, it does describe those which would be of major use in the conduct of the proposed research and demonstrates that ITRI has all critical resources necessary to meet the needs of this research proposal. Further, the essential resources will be available on a noninterference basis relative to our commitments to DOE and other federal agencies currently supporting research at ITRI.

XIII. QUALITY ASSURANCE

The research described in this proposal is exploratory in nature and will be conducted in the spirit of the Good Laboratory Practice (GLP) regulations. Specific quality requirements will be addressed as integral parts of project-specific protocol(s) and standard operating procedures that might be needed. The approval of the ITRI Quality Assurance Unit is required on all protocols and standard operating procedures at the ITRI.

General requirements for research conducted in the spirit of GLPs at ITRI are that study personnel will have documented training and experience; that work will be done following ITRI-approved protocols and applicable ITRI-approved standard operating procedures; that study personnel follow good recordkeeping procedures and verify the accuracy of data collected; that personnel maintain calibration and maintenance documentation for equipment and instruments; and that study personnel ensure test chemicals, reagents, and specimens integrity throughout collection, analysis' and storage processes.

It has been our experience that research such as this is difficult to conduct within strict GLP guidelines due to the many adjustments that occur in the research plan as protocols are developed and conducted.

API staff, representatives of sponsoring companies, and consultants retained by API, will have reasonable access to LBERI personnel and facilities engaged in the work covered by this Agreement.

API may designate one or more consultants who may observe or audit the conduct of the work by this proposal. The LBERI shall cooperate fully with such observations and audits.

All records, new data or other documentation relating to the studies covered by this Agreement shall be retained by LBERI according to GLP regulations for a period of at least ten (10) years from the date of conclusion of the studies.

The materials retained pursuant to the above paragraph shall be stored in an archive which ensures that they will be maintained in a safe and secure manner and allows for their expeditious retrieval when needed. Material retained in the archive shall be indexed by test substance, date of study, test system and nature of study. In the event that the Contractor or its archiving facility discontinue operations or for other reasons are not able to archive the material, all raw data, specimens and other documentation pertaining to the testing covered by this Agreement shall be transferred to such other facility as API may direct.

XIV. HEALTH PROTECTION

All applicable ITRI health and safety requirement will be adhered to. These cover general laboratory practices. Specific safety requirements will be included as an integral part of the protocols and standard operating procedures required for the studies. The approval of the ITRI Health Protection Operations Unit is required on all protocols and standard operating procedures at the ITRI.

XV. CARE AND USE OF LABORATORY ANIMALS

The use of laboratory animals in well-designed biomedical research studies has resulted in significant advances in health care and prevention of disease in humans. Animals are used at the ITRI in well-defined studies to identify the health risks to man from exposure to potentially toxic agents in the absence of an adequate understanding of their effects on

man. Other methods such as cell culture are used whenever possible to reduce the number of animals needed in the studies. However, adequate alternatives to the use of laboratory animals for the studies proposed here are not currently available. As such, the use of laboratory animals for these studies is necessary.

We recognize that the use of animals in studies such as these is a privilege, not a right. Thus, policies have been instituted to insure the ethical and humane care and treatment of animals at the ITRI. This is accomplished through review and approval of protocols using animals and the establishment of standard operating procedures for their care. ITRI has a current National Institutes of Health assurance number for animal care and use; that number is A3083-01. ITRI is also fully accredited by the American Association for Accreditation of Laboratory Animal Care; our accreditation number is 000200. ITRI has an officially constituted "Animal Care and Use Committee"; the responsibilities of the Committee include reviewing all animal care and usage at ITRI. The Committee is responsible for assuring that all animals at ITRI are kept, treated, and used in a humane and lawful manner. This draft proposal is currently under review by the ITRI Animal Research Committee and will be approved prior to final submission to API. ITRI is a registered research facility with the USDA; our registration number 85-R-003. ITRI is in full compliance with the Animal Welfare Act as administered by the USDA. ITRI is in full compliance with all other relevant local, state, and federal regulations concerning animal care and use.

XVI. PROJECT COSTS

A. General

The costs given in Table 2 are our best estimates of the costs for conducting the research outlined in the proposal. Because of uncertainties of the exact protocols that will be used, the budget may require modification as the protocols are finalized or as the direction of the research is modified from the findings in early stages of the research.

The ITRI is operated by the Lovelace Biomedical and Environmental Research Institute (LBERI) for the DOE. The LBERI is a non-profit corporation which operates on a cost-reimbursable no-fee basis. Therefore, the costs for performing the research will in all cases be the actual costs (labor, supplies, services, etc.) of the effort expended to do the work. The ITRI cannot exceed the amount of the agreement without amending the agreement through the DOE and with the sponsor.

B. Basis for Cost Estimates

The cost estimates in Table 2 are for the level of effort stated and our best estimate of the cost of supplies required to do the studies outlined in the proposal. The salaries used are those projected to be in effect for the period 1 Jan 1993 through 31 Dec 1996 (3 years).

As work progresses, we reserve the right to change personnel assignments and to execute procurement actions based on research results obtained, the nature of proposed research, and our considered scientific and managerial judgment. Procurement activities will be carried out in accordance with DOE procurement regulations in effect at the time purchases are made.

Table 2

Projected Cost Estimate for
Biomarkers of Exposure to Benzene

	Year 1 Effort	Year 1 $	Year 2 Effort	Year 2 $	Year 3 Effort	Year 3 $	Total Effort	Total $
PERSONNEL								
R. Henderson, Principal Investigator	.10		.10		.10		.30	
W. Bechtold, Chemist	.10		.20		.10		.40	
I. Chang, Statistician	.20		.00		.10		.30	
To be Assigned, Research Technologists	.10		.30		.20		.60	
Total Salary		24,900		29,200		25,400		79,500
Fringe Benefits		6,000		7,000		6,100		19,100
Supplies/Services								
Animal Purchase		1,000		1,000		0		2,000
Miscellaneous supplies/glassware		10,000		10,500		11,000		31,500
Subtotal		11,000		11,500		11,000		33,500
Travel		1,000		1,500		2,000		4,500
Other Expenses								
Animal maintenance		1,200		1,200		0		2,400
Word processing service		500		500		500		1,500
Illustration service		500		500		500		1,500
Computer service		5,000		5,300		5,600		15,900
Subtotal		7,200		7,500		6,600		21,300
Total Direct Costs		50,100		56,700		51,100		157,900
Indirect Costs		35,100		39,700		35,800		110,600
Total Costs		85,200		96,400		86,900		268,500

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The fringe benefit rate is calculated as a percent of total salaries and includes the following:

FICA

Medical Insurance

Retirement Benefits

Life Insurance

Disability Insurance

Unemployment Compensation

Workmen's Compensation

This rate has had an increasing trend, attributable to increases in the employer-paid FICA rate as well as medical insurance. The 24 percent rate used is based on the last three years' operating experience.

The overhead costs include those costs associated with functions such as general management and administration, business office, procurement, property management, personnel, facilities operation and maintenance, library, quality assurance, and health protection operations.

The Institute's overhead cost rate is calculated and adjusted monthly with an annual rate set at the end of each fiscal year. All costs incurred are audited normally by U. S. Government auditors, and all overhead rates are verified. The last fiscal year audited was FY-1991 and, as in previous years, no exceptions in rates were noted.

The Institute's overhead expenses are distributed as a percentage rate on all modified total direct costs. Although the rate in FY-1992 was lower than what we are projecting for FY-1993, it is a provisional rate and actual charges will be made.

In-house support services include the costs of animals, animal care, histopathology preparation, shop, computer, word processing and illustration services that are provided within the Institute on a centralized basis. The costs of these services are charged to direct projects on a pro rata basis dependent upon the level of service used by each individual project. The number of animals used and the days of animal maintenance have been based on the proposed work plan. The level of shop, computer, word processing and illustration services required for this project were estimated based on our experience with similar projects.

Travel expenses are for investigators to present results at national meetings and to report results at meetings called by the API.

The estimated budget is written for a 3-year period even though we realized that the initial contract will be for one year only.

Draft Proposal

for the Study of

DOSIMETRY OF BENZENE IN THE BONE MARROW

Submitted to the

American Petroleum Institute

by the

Inhalation Toxicology Research Institute

P. O. Box 5890

Albuquerque, NM 87185

Operated by the

Lovelace Biomedical and Environmental Research Institute

for the

U. S. Department of Energy

under Contract No. DE-AC04-76EV01013

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ITRI Contact:

C. H. Hobbs, DVM
ITRI Assistant Director
505-845-1045

or

R. F. Henderson, PhD
Principal Investigator
505-845-1154

I. INTRODUCTION

Benzene is a known hematotoxin and leukemogen in humans. To determine the health risks associated with benzene exposure in a quantitative fashion, it is essential to determine dose/response relationships. The dose of greatest interest is the biologically effective dose, i.e., the dose that causes a change in the biological system that leads to an adverse health effect. In the case of benzene, the mechanism by which benzene and/or its metabolites induces acute myelogenous leukemia in humans is not known. In the absence of a mechanism, one cannot determine the true biologically effective dose, but the dose to the target tissue, the bone marrow, can be determined. Recent work by Dr. Richard Irons implicates the benzene metabolite, hydroquinone, as a major toxic benzene metabolite in the bone marrow (Irons *et al.*, 1992). Other work implicates free radical forms of benzene metabolites as potential bone marrow toxins (Subrahmanyam *et al.*, 1991). The following proposal outlines research to be conducted in collaboration with Dr. Irons to determine the dosimetry of benzene and its metabolites in the bone marrow that is associated with specific toxic endpoints.

II. BACKGROUND

Reports by Irons (1992) indicate that *in vitro* pretreatment of mouse bone marrow cells with pmolar levels of hydroquinone greatly enhances the response of the cells to granulocyte/macrophage colony-stimulating factor (rGM-CSF). No stimulation occurred with phenol, catechol or muconaldehyde. The bone marrow cells affected by the hydroquinone and rGM-CSF were nonadherent and lineage-depleted bone marrow cells, suggesting an

intrinsic effect on recruitment of myeloid progenitor cells, cells not normally responsive to rGM-CSF.

In *in vivo* experiments, Eastmond, Smith and Irons reported a synergistic effect of co-administration of phenol and hydroquinone in inducing hematotoxic effects in mice. The authors suggested that the effect was due to phenol stimulation of peroxidase-dependent metabolism of hydroquinone to form the highly reactive benzoquinone.

Growing evidence supports the contention that free radicals may play a role in inducing the toxic effects of benzene (Subrahmanyam *et al.*, 1991). It has been suggested that either myeloperoxidase, prostaglandin (H) synthase, or eosinophil peroxidase enzymes react with the phenolic metabolites of benzene to produce free radical intermediates. These free radical intermediates can then initiate lipid peroxidation, deplete cellular antioxidants such as glutathione, or cause damage to DNA and proteins. Evidence from both *in vivo* and *in vitro* studies suggests that these events do occur as a result of benzene metabolism.

For instance, Lewis *et al.* (1988) showed that of the quinone metabolites of benzene, only hydroquinone and benzenetriol were capable of auto-oxidizing to produce superoxide anion free radicals, and only these two metabolites were effective at causing DNA single-strand breaks. However, no *in vivo* data has been reported that links the dose of the metabolite to the target cells with the subsequent induction of DNA strand breaks.

In an *in vivo* study, Anwar *et al.* (1989) showed that dimethyl sulfoxide (DMSO, a hydroxyl radical scavenger) could reduce by 95% the micronuclei frequency in bone marrow of mice dosed orally with benzene when administered by gavage 1 hr after the benzene exposure. This reduction in micronuclei frequency was dependent on the DMSO concentration. However, the treatment with DMSO also reduced the levels of urinary

benzene metabolites. This finding raises the question as to whether the DMSO altered the normal uptake of benzene to yield a lower dose of benzene and its metabolites to the target tissue, bone marrow, or whether DMSO protected the target tissue by scavenging free radicals. When only one free radical scavenger is used, it is difficult to establish that this agent acted only as a free radical scavenger, and that its effect was not due to some other property of the agent. It is important to establish that the concentration of the scavenger in the target tissue was adequate to perform a free radical scavenging function. It is equally important to establish that the scavenger did not alter the normal metabolism and distribution of the benzene metabolites.

The ITRI has had extensive experience in determining the effect of exposure concentration, species and route of exposure on the metabolism of benzene. Work completed in $B6C3F_1$ mice, F344/N rats and cynomolgus monkeys indicates that the formation of hydroquinone and muconaldehyde metabolites are favored at low doses or exposure concentrations (Henderson *et al.*, 1989; Sabourin *et al.*, 1989; Medinsky *et al.*, 1989; Sabourin *et al.*, 1992). For inhalation exposures, the formation of hydroquinone metabolites or muconic acid is linearly related to exposure concentration up to 200 ppm benzene (6-hr exposures), but decreases at higher concentrations. The relation of exposure concentration to blood and urine levels of the major benzene metabolites has been determined based on the "area under the curve" for each metabolite during and after the 6-hr exposure. However, equivalent values have not been obtained for bone marrow, for which only pooled samples (pooled samples from all time points during and after the exposure) were analyzed.

III. SPECIFIC AIMS

The specific aims of this project are:

1. To determine the relationship between exposure concentration and the dosimetry of benzene and its metabolites in the bone marrow of $B6C3F_1$ mice and to correlate the dosimetry with toxic changes in the marrow.
2. To determine the role of free radicals in producing toxicity in the bone marrow of benzene-exposed mice.

IV. PROPOSED EXPERIMENTS

A. Specific Aim 1

A series of studies designed to link *in vivo* dosimetry data for benzene and its metabolites to the potential biological effects described above are planned. Prior work at the ITRI has indicated that the pathways leading to putative toxic metabolites of benzene (represented by hydroquinone metabolites and muconic acid) are saturated at low exposure concentrations, so that a higher fraction of the metabolites are related to putative toxic metabolites at low doses than at high doses (Henderson *et al.*, 1989; Sabourin *et al.*, 1989). The formation of hydroquinone metabolites and muconic acid are linearly related to exposure concentration up to about 200 ppm (6-hr exposure), but decrease as a fraction of the total metabolites formed above that concentration. We wish to choose two exposure concentrations below this breakpoint and one above it, to see the effect of concentration on dosimetry to the bone marrow and on the induced toxic effects. $B6C3F_1$ mice will be exposed for 6 hr to either filtered air or 6, 60, or 600 ppm benzene. The levels of benzene and benzene metabolites will be determined in the blood, bone marrow, and urine during (at 2 and at 4 hr)

and after the exposure (up to 12 hr) to obtain an "area under the curve" dosimetry for each metabolite. The dosimetry of the metabolites will be compared to those required to induce toxic changes in the bone marrow in Irons' *in vitro* studies. Toxic responses in the bone marrow will be observed by Dr. Irons. The effects of benzene exposure *in vivo* will be examined on colony-forming response of murine bone marrow cells stimulated with recombinant cytokines. Mice will be killed by cervical dislocation and bone marrow flushed from femora using phosphate-buffered saline containing 1% bovine serum albumin. Bone marrow cells will be purified over a discontinuous gradient and nonadherent cells obtained. Purified cell suspensions will be transported on ice to Dr. Irons' laboratory where they will be cultured as previously described (Irons *et al.*, 1992).

It is recognized that the optimum time of exposure for inducing toxic effects is not known. Therefore, the exposure time may be varied to achieve a maximal response if that is necessary. The exposure time of 6 hr is based on a large data base at ITRI based on 6-hr exposures. This data base indicates that blood metabolites have essentially come to equilibrium by 6 hr of exposure of mice to as low as 5 ppm benzene.

B. Specific Aim 2

In order to assess whether DMSO acts as a scavenger of benzene-induced free radicals or by altering benzene metabolism or distribution, one set of animals will be dosed with DMSO one hour after benzene exposure. In addition, we will examine mice that have been treated with buthionine sulfoximine (BSO), a glutathione synthesis inhibitor. If free radicals are involved in benzene toxicity, depleted glutathione induced by BSO treatment should exacerbate benzene toxicity. Glutathione depletion should not significantly affect benzene metabolite levels as glutathione conjugation is only a minor pathway for benzene

metabolism in mice (Sabourin *et al.*, 1989). Thus, if free radical intermediates are involved in benzene-induced toxicity, DMSO should mitigate the toxicity and BSO should exacerbate the toxicity. Both dosimetry (ITRI) and bone marrow toxicity (Irons) will be evaluated in the mice.

Besides being a scavenger of free radicals, DMSO may also alter membrane characteristics, especially at the high concentrations of DMSO used in many *in vitro* studies. Such alterations of membranes would complicate interpretation of results in our studies. We anticipate that tissue levels of DMSO will be too low *in vivo* to cause such complications. We plan to assay for DMSO in the tissues of treated mice to address this issue.

For DMSO-treated mice, one hr after exposure DMSO (12.5% at a volume of 0.01 ml/g body weight) will be administered by gavage to one set of mice in each exposure group (controls and exposed groups). For BSO-treated mice, one set of both controls and exposed mice will be administered BSO in their drinking water prior to exposure. The glutathione levels in the bone marrow of these mice will be examined immediately before and after the benzene exposure to determine whether the BSO treatment did indeed deplete bone marrow glutathione levels. After exposure, mice from all groups will be placed in metabolism cages and urine will be collected for 24 hr. At the end of this period, the mice will be sacrificed. The pooled urine, the blood, and the bone marrow will be analyzed for hydroquinone, phenol, and catechol by the method of Rickert *et al.* (1979), for conjugated benzene metabolites by the method of Sabourin *et al.* (1989), and for DMSO using a GC/MS technique. The bone marrow tissue will also be assayed for micronuclei formation as described in the aforementioned paper by Anwar *et al.* (1989) and for DNA single strand breaks using standard techniques.

C. Time Line

The time line for the expected completion of these studies is shown in Figure 1.

V. EXPECTED RESULTS

These studies will integrate dosimetry studies with studies that determine the effects of that dosimetry on the bone marrow. The results will provide information on which metabolites are the likely biologically effective compounds and if free radicals are involved in producing the toxic responses.

VI. FUTURE PLANS

If desired by API, a similar approach can be used to study the effect of co-exposure to toluene on the dosimetry of benzene and its metabolites in bone marrow. An inhibitory effect of toluene on the metabolism of benzene has been reported by Ikeda *et al.* (1972).

VII. REFERENCES

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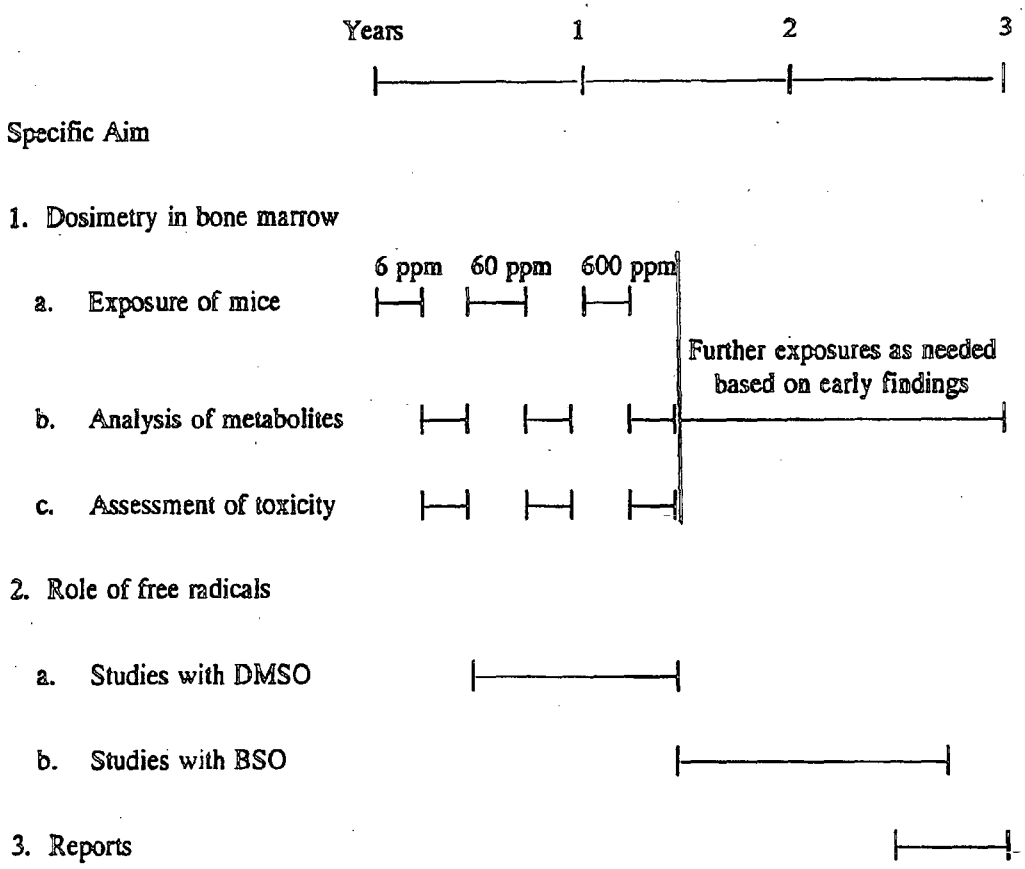

Figure 1. Time Line for Proposed Study

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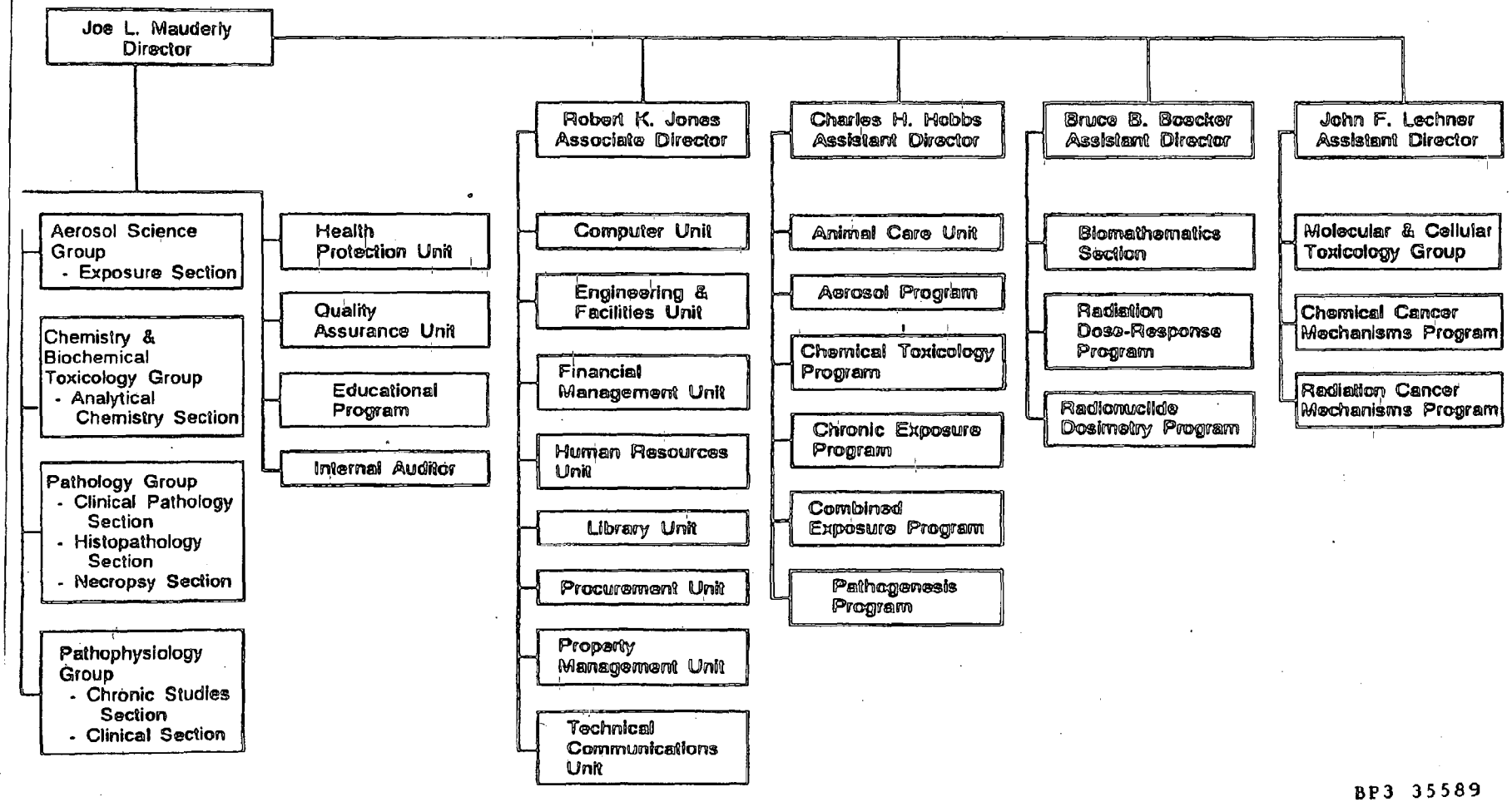
VIII. RESOURCE AND RESEARCH MANAGEMENT

The Institute's resource management structure is shown in Figure 2. ITRI's Director, Dr. Joe L. Mauderly, serves also as President of LBERI. Dr. Robert K. Jones is the Associate Director of ITRI and Vice-President of LBERI. Dr. Charles H. Hobbs, Dr. John F. Lechner and Dr. Bruce B. Boecker act as Assistant Directors of ITRI. Dr. Hobbs is responsible for management of the chemical toxicology projects at ITRI, Dr. Lechner is responsible for molecular biology projects, and Dr. Boecker is in charge of projects in radiation toxicology. These five members of the Directorate provide overall institutional resource management and each has responsibility for people, space and equipment included within their specific research groups and support service units. The research project described in this proposal will be under the overall supervision of Dr. C. H. Hobbs. Each project also has a project coordinator or study director. In this case it will be Dr. R. F. Henderson.

Since the Institute is operated under contract with the DOE, we must and do comply with all federal regulations relative to personnel activities, procurement, property management, accounting standards, facility maintenance, security and health and safety procedures. We have developed and utilize a computerized management information system

FIGURE 2

INHALATION TOXICOLOGY RESEARCH INSTITUTE
Organizational Structure



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to account for all financial transactions and manpower data as well as for activities relating to property management and other support services activities. Monthly printed reports on current period and year-to-date transactions are provided to all appropriate personnel for management purposes. Finally, it should be noted that a year-end financial audit is conducted on the Institute's prior fiscal year financial transactions by U. S. Government auditors to assure compliance with federal regulations and to verify an appropriate indirect cost basis which is applicable to all sponsors for whom research has been conducted. In summary, we feel that the Institute has the effective and experienced management team required to conduct both cost-effective and high-quality research for sponsors.

IX. RESEARCH PROJECT MANAGEMENT AND PERSONNEL

A multidisciplinary team of scientists has been chosen for work on this project. Table 1 lists the members of the research team. Curriculum vitae of all professional staff are included in the Appendix.

The ITRI scientists with primary responsibility for the management and conduct of these proposed studies are Dr. C. H. Hobbs and Dr. R. F. Henderson. In the context of the Good Laboratory Practice Regulations, Dr. Henderson will be the Study Director (Principal Investigator) for the studies and as such she is responsible for overall conduct of the studies and will serve as the contact at ITRI for API for conducting the studies. Dr. Henderson is trained as a biochemical toxicologist and has extensive experience in the toxicokinetics of inhaled vapors.

Dr. Kirk Maples is a chemist and will be in charge of monitoring the exposures and analyzing samples for benzene and its metabolites.

Table 1

Summary of Scientific Personnel with Substantial Involvement in the Project

Name	Degree/Discipline	Role in Study
Henderson, R. F.	Ph.D., Biochemical Toxicologist	Study Director (Principle Investigator)
Maples, K. R.	Ph.D., Chemist, Toxicologist	Co-Investigator, Metabolite Analysis
Chang, I.-Y.	M.S., Statistician	Co-Investigator, Statistical Analyses

Dr. I-Yiin Chang is a statistician with experience in analysis of animal toxicology data. She will be responsible for all statistical analyses of data on the effect of exposure concentration on the level of benzene metabolites, tissues, and excreta samples.

Appropriate technical and other supporting personnel will be assigned to the project to meet project objectives. The professional, technical and other supporting personnel will work as a team. Should the need arise for technical expertise not available from personnel on the team, assistance of other members of the Institute staff will be enlisted. The Institute retains the authority to assign equivalent personnel or otherwise use Institute personnel, within the limits of the authorized budget, to best achieve the project's objectives.

X. REPORTS

At 6-mo intervals, a letter report on the progress of the project will be submitted to API by LBERI.

The LBERI will immediately notify API of any private or governmental request for information on ongoing or completed research conducted under this Agreement, including any subpoena or other legal instrument requesting information. The Contractor will cooperate fully with any effort by API to narrow the scope of any such request, to obtain a protective order limiting the use or disclosure of information.

XI. PUBLICATIONS

API and the LBERI agree to full public disclosure of scientific information developed through this Agreement. The LBERI shall not release such information without prior API knowledge and review.

The scientific conclusions and professional judgments arising out of performance of the project will be the responsibility of the LBERI. The LBERI, however, will not publish or otherwise release data, conclusions, or manuscripts in a citable or quotable form without prior API knowledge and opportunity to comment. API opportunity to comment is for the purpose of clarification and format or editorial comments, but not for the purpose of substituting API's opinion for that of the LBERI.

XII. INSTITUTIONAL RESOURCES

A. General

The Inhalation Toxicology Research Institute (ITRI) has been operated by the Lovelace organization for the U. S. Department of Energy (DOE) or its predecessor agencies since the early 1960's. It is currently operated under a DOE five-year, cost-reimbursable, no-fee contract by the Lovelace Biomedical and Environmental Research Institute (LBERI), a subsidiary of the Lovelace Medical Foundation (LMF). LBERI's only mission is to operate ITRI for DOE and to conduct research for DOE and other sponsors where appropriate. LBERI has no financial assets other than those it is responsible for managing for the United States government or its other sponsors.

ITRI facilities are totally government owned and are located on Kirtland Air Force Base East in Albuquerque, New Mexico. These facilities contain approximately 271,000 ft^2 of space for laboratories, animal breeding and maintenance activities, offices and essential support services. Since first occupied in 1963, new additions or modifications have been added on nearly an annual basis to meet the ever-changing nature of the Institute's research program. These facilities represent one of the most modern and complete

laboratories for the conduct of inhalation toxicology research that are available anywhere in the world. Pertinent details are provided below.

B. Facilities

The Institute's government-owned facilities consist of approximately 271,000 ft^2 of space. They include a central laboratory, office and small animal and primate maintenance complex, dog kennels on either side of the central laboratory and specialized facilities for machine shop, electronics shop, analytical chemistry, rodent quarantine and rodent breeding, primate quarantine, receiving and warehouse, health protection and waste handling located more peripheral to the central complex.

From the standpoint of this proposal, the following facilities are considered to be of principal importance:

1. Modern analytical chemistry laboratories containing state-of-the-art instruments. The instrumentation includes liquid scintillation counters, spectrofluorometers, high-pressure liquid chromatographs with auto injectors and auto sample collectors, UV and VIS spectrophotometers, gas chromatograph/mass spectrometers with a computer data system, gas chromatographs, a laboratory data system for quantitation of GC and HPLC data, infrared spectrometer with a computerized data system, and a nuclear magnetic resonance spectrometer.
2. Laboratories for safely developing and testing generation procedures for inhalation exposure environments using either radiolabeled or unlabeled materials, including potential carcinogens. These laboratories are

equipped with instruments for physicochemical characterization of the exposure environments.

3. Hazardous material storage and preparation area designed and constructed for storage of hazardous materials in a controlled access area. The facility has storage capabilities adjacent to a preparation area. This area provides for storage of radioactive materials in a shielded vault and storage of nonradioactive materials in a cabinet or refrigerator. The preparation room is equipped with a hot cell and three hoods for preparing aerosol generator solutions and other operations related to preparing materials for use in inhalation exposures.

4. Inhalation exposure facilities specifically designed to allow controlled access to an area fully equipped for acute inhalation exposures. Each of two exposure complexes within the facility has its own control room, two exposure rooms and a preparation room. Associated equipment in the facility includes an ultrasonic unit for decontamination of exposure apparatus and an electron microscope. A variety of laboratory animals have been exposed nose only in the facility, ranging in size from mice to dogs. Materials aerosolized for animal exposures have included chemical carcinogens, a variety of radioactive compounds and vapors. Exposures have included respiratory monitoring by whole-body plethysmography in some cases and the facility is equipped to make these respiratory measurements for small laboratory animals, dogs and monkeys.

5. Quarantine facilities are available for isolation and stabilization of animals received from commercial suppliers. Rigid standard operating procedures are followed during the quarantine period while the health status of newly acquired animals is evaluated.

6. A barrier-sustained, rodent housing facility is operated on a clean-dirty corridor system. All personnel must shower before entering the facility and must wear clean laboratory-supplied clothing, face mask and gloves. All materials entering the facility are sanitized (i.e., cages, lids, caps, personnel clothing). Feed is pasteurized by suppliers and all bedding is sterilized prior to use. All items that cannot be entered through the tunnel washer or autoclaved are chemically sterilized. The animal rooms are maintained at 20 to 23°C and at 30 to 50% relative humidity under a 12 hr light and dark cycle.

ITRI is a registered research facility, in compliance with Section 6 of P.L. 89-544 (as amended), the Laboratory Animal Welfare Act, and has been fully accredited by the American Association for Accreditation of Laboratory Animal Care since 1970. The last accreditation inspection was conducted in June, 1988. Animals are maintained in accordance with the recommendations in NIH Publication No. 85-23, entitled "Guide for the Care and Use of Laboratory Animals" and in compliance with the Laboratory Animal Welfare Act.

7. A cellular toxicology facility is specifically designed and staffed to test the effects of chemical toxicants, carcinogens and radiation on

eukaryotic and prokaryotic cells in culture. Equally important are the capabilities in this facility to determine the *in vitro* functional capacities of cells exposed to toxicants *in vivo*. A FACSTAR PLUS (Becton-Dickinson) flow cytometer is available.

8. A comprehensive program for hazardous waste handling and disposal. Radioactive and hazardous wastes generated at the Institute are collected, treated, packaged and shipped in accordance with all EPA, DOT and DOE regulations, and in a manner that minimizes hazard to personnel or the environment.

   Hazardous chemical wastes are collected and handled in a manner consistent with the requirements of the Resource Conservation and Recovery Act (RCRA). As with radioactive wastes, all hazardous chemical wastes are shipped to EPA-approved disposal sites for final disposal.

9. In-house computer capability consisting of a network of Digital Equipment Corporation VAX series computers. It currently includes a VAX 11/780, three MicroVAX II computers and five PDP-11 series computers. The latter are required for real-time data acquisition. Extensive use is also made of personal computers for automated data collection, scientific calculations, word processing and graphics. These computer resources provide facilities for collection, storage and processing of both scientific and business or management data for the total Institute.

10. Emergency electrical generation capability is provided to all essential areas of the facilities. This capability includes two diesel-powered 750 KW and two 100 KW generators. They receive routine testing on a weekly basis and require only six seconds for full activation. All of the facilities described above are incorporated into this emergency power grid.

11. A complete in-house shop capability including a machine shop, electronics shop and carpenter shop. The personnel in each shop area continue to provide the necessary experience and skills for the initial design and fabrication or subsequent modification of prototype exposure chambers, characterization devices and other instruments required at ITRI.

12. An excellent research library which provides all services required to support our research program. These include current subscription to 300 journals, over 9,500 books and approximately 19,000 documents. Local and regional interlibrary loan services are available through the University of New Mexico and a consortium of New Mexico Medical Libraries. On-line literature search services are maintained with over 100 data bases through 4 major systems. These services will be essential for providing available literature on specific chemicals which ITRI is requested to study.

Although this by no means describes the Institute's total facility and equipment resources, it does describe those which would be of

major use in the conduct of the proposed research and demonstrates that ITRI has all critical resources necessary to meet the needs of this research proposal. Further, the essential resources will be available on a noninterference basis relative to our commitments to DOE and other federal agencies currently supporting research at ITRI.

XIII. QUALITY ASSURANCE

The research described in this proposal is exploratory in nature and will be conducted in the spirit of the Good Laboratory Practice (GLP) regulations. Specific quality requirements will be addressed as integral parts of project-specific protocol(s) and standard operating procedures that might be needed. The approval of the ITRI Quality Assurance Unit is required on all protocols and standard operating procedures at the ITRI.

General requirements for research conducted in the spirit of GLPs at ITRI are that study personnel will have documented training and experience; that work will be done following ITRI-approved protocols and applicable ITRI-approved standard operating procedures; that study personnel follow good recordkeeping procedures and verify the accuracy of data collected; that personnel maintain calibration and maintenance documentation for equipment and instruments; and that study personnel ensure test chemicals, reagents, and specimens integrity throughout collection, analysis' and storage processes.

It has been our experience that research such as this is difficult to conduct within strict GLP guidelines due to the many adjustments that occur in the research plan as protocols are developed and conducted.

API staff, representatives of sponsoring companies, and consultants retained by API will have reasonable access to LBERI personnel and facilities engaged in the work covered by this Agreement.

API may designate one or more consultants who may observe or audit the conduct of the work by this proposal. The LBERI shall cooperate fully with such observations and audits.

All records, new data or other documentation relating to the studies covered by this Agreement shall be retained by LBERI according to GLP regulations for a period of at least ten (10) years from the date of conclusion of the studies.

The materials retained pursuant to the above paragraph shall be stored in an archive which ensures that they will be maintained in a safe and secure manner and allows for their expeditious retrieval when needed. Material retained in the archive shall be indexed by test substance, date of study, test system and nature of study. In the event that the Contractor or its archiving facility discontinue operations or for other reasons are not able to archive the material, all raw-data, specimens and other documentation pertaining to the testing covered by this Agreement shall be transferred to such other facility as API may direct.

XIV. HEALTH PROTECTION

All applicable ITRI health and safety requirement will be adhered to. These cover general laboratory practices. Specific safety requirements will be included as an integral part of the protocols and standard operating procedures required for the studies. The approval of the ITRI Health Protection Operations Unit is required on all protocols and standard operating procedures at the ITRI.

XV. CARE AND USE OF LABORATORY ANIMALS

The use of laboratory animals in well-designed biomedical research studies has resulted in significant advances in health care and prevention of disease in humans. Animals are used at the ITRI in well-defined studies to identify the health risks to man from exposure to potentially toxic agents in the absence of an adequate understanding of their effects on man. Other methods such as cell culture are used whenever possible to reduce the number of animals needed in the studies. However, adequate alternatives to the use of laboratory animals for the studies proposed here are not currently available. As such, the use of laboratory animals for these studies is necessary.

We recognize that the use of animals in studies such as these is a privilege, not a right. Thus, policies have been instituted to insure the ethical and humane care and treatment of animals at the ITRI. This is accomplished through review and approval of protocols using animals and the establishment of standard operating procedures for their care. ITRI has a current National Institutes of Health assurance number for animal care and use; that number is A3083-01. ITRI is also fully accredited by the American Association for Accreditation of Laboratory Animal Care; our accreditation number is 000200. ITRI has an officially constituted "Animal Care and Use Committee"; the responsibilities of the Committee include reviewing all animal care and usage at ITRI. The Committee is responsible for assuring that all animals at ITRI are kept, treated, and used in a humane and lawful manner. This draft proposal is currently under review by the ITRI Animal Research Committee and will be approved prior to final submission to API. ITRI is a registered research facility with the USDA; our registration number 85-R-003. ITRI is in full compliance with the Animal

Welfare Act as administered by the USDA. ITRI is in full compliance with all other relevant local, state, and federal regulations concerning animal care and use.

XVI. PROJECT COSTS

A. General

The costs given in Table 2 are our best estimates of the costs for conducting the research outlined in the proposal. Because of uncertainties of the exact protocols that will be used, the budget may require modification as the protocols are finalized or as the direction of the research is modified from the findings in early stages of the research.

The ITRI is operated by the Lovelace Biomedical and Environmental Research Institute (LBERI) for the DOE. The LBERI is a non-profit corporation which operates on a cost-reimbursable no-fee basis. Therefore, the costs for performing the research will in all cases be the actual costs (labor, supplies, services, etc.) of the effort expended to do the work.

B. Basis for Cost Estimates

The cost estimates in Table 2 are for the level of effort stated and our best estimate of the cost of supplies required to do the studies outlined in the proposal. The salaries used are those projected to be in effect for the period 1 Jan 1993 through 31 Dec 1996 (3 years).

As work progresses, we reserve the right to change personnel assignments and to execute procurement actions based on research results obtained, the nature of proposed research, and our considered scientific and managerial judgment. Procurement activities will be carried out in accordance with DOE procurement regulations in effect at the time purchases are made.

Table 2

Projected Cost Estimate for
Dosimetry of Benzene in the Bone Marrow

	Year 1		Year 2		Year 3		Total	
	Effort	$	Effort	$	Effort	$	Effort	$
PERSONNEL								
R. Henderson, Principal Investigator	.15		.15		.15		.45	
K. Maples, Chemist	.20		.20		.20		.60	
I. Chang, Statistician	.05		.05		.05		.15	
To be Assigned, Research Technologists	.40		.40		.40		1.20	
Total Salary		36,800		38,600		40,500		115,900
Fringe Benefits		9,200		9,700		10,100		29,000
Supplies/Services								
Animal Purchase		1,000		1,100		1,100		3,200
Miscellaneous supplies/glassware		15,000		15,500		16,000		46,500
Subtotal		16,000		16,600		17,100		49,700
Travel		3,000		3,200		3,400		9,600
Other Expenses								
Animal maintenance		600		600		600		1,800
Word processing service		500		1,100		1,200		2,800
Illustration service		500		1,100		1,200		2,800
Computer service		1,000		1,100		1,200		3,300
Subtotal		2,600		3,900		4,200		10,700
Total Direct Costs		67,600		72,000		75,300		214,900
Indirect Costs		47,300		50,400		52,700		150,400
Total Costs		114,900		122,400		128,000		365,300

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The fringe benefit rate is calculated as a percent of total salaries and includes the following:

FICA

Medical Insurance

Retirement Benefits

Life Insurance

Disability Insurance

Unemployment Compensation

Workmen's Compensation

This rate has had an increasing trend, attributable to increases in the employer-paid FICA rate as well as medical insurance. The 24 percent rate used is based on the last three years' operating experience.

The overhead costs include those costs associated with functions such as general management and administration, business office, procurement, property management, personnel, facilities operation and maintenance, library, quality assurance, and health protection operations.

The Institute's overhead cost rate is calculated and adjusted monthly with an annual rate set at the end of each fiscal year. All costs incurred are audited normally by U. S. Government auditors, and all overhead rates are verified. The last fiscal year audited was FY-1991 and, as in previous years, no exceptions in rates were noted.

The Institute's overhead expenses are distributed as a percentage rate on all modified total direct costs. Although the rate in FY-1992 was lower than what we are projecting for FY-1993, it is a provisional rate and actual charges will be made.

In-house support services include the costs of animals, animal care, histopathology preparation, shop, computer, word processing and illustration services that are provided within the Institute on a centralized basis. The costs of these services are charged to direct projects on a pro rata basis dependent upon the level of service used by each individual project. The number of animals used and the days of animal maintenance have been based on the proposed work plan. The level of shop, computer, word processing and illustration services required for this project were estimated based on our experience with similar projects.

Travel expenses are for investigators to present results at national meetings and to report results at meetings called by the API.

The estimated budget is written for a 3-year period even though we realized that the initial contract will be for one year only.