NCTR
Research Accomplishments and Plans

FY 1998-1999

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PREFACE

The National Center for Toxicological Research (NCTR), one of the research facilities within the Jefferson Laboratories of the Food and Drug Administration (FDA), is located in Jefferson, a rural community in south-central Arkansas, approximately 30 miles from Little Rock. The Jefferson Laboratories of the FDA is comprised of both the NCTR and the Arkansas Regional Laboratory (ARL) of the Office of Regulatory Affairs (ORA).

The mission of the NCTR is to conduct peer-reviewed scientific research that supports and anticipates the FDA's current and future regulatory needs. This involves fundamental and applied research specifically designed to define biological mechanisms of action underlying the toxicity of products regulated by the FDA. This research is aimed at understanding critical biological events in the expression of toxicity and at developing methods to improve assessment of human exposure, susceptibility and risk.

NCTR conducts integrated research with other FDA centers/Office of Regulatory Affairs and leverages FDA resources through cooperative and/or collaborative agreements with other agencies, academia and industry. These interactions enhance opportunities to provide more effective risk measures for FDA-regulated products and support FDA enforcement through methods development.

NCTR research is focused within three strategic research goals:

The development of new strategies for the prediction of toxicity (PRED) based on mechanism-based assays that contribute to a profile of information that supports a regulatory decision.

The development of computer-based systems that predict human toxicity (knowledge bases) (KNLG) by taking advantage of NCTR core capabilities in fundamental and applied research to build knowledge bases that will support more accurate assessment of human toxicity and risk.

The conduct of method- (METH), agent- (AGNT), or concept-driven (CNPT) research to provide data on specific agents of concern to the FDA; develop analytical and toxicological test methods to improve the FDA's post-market surveillance capability; and conduct studies designed to better understand the mechanisms of toxicity and carcinogenicity in both animal models and epidemiological studies used to identify toxicity.

NCTR research is conducted within seven research divisions whose goals, ongoing research accomplishments and FY99 plans are summarized herein. All NCTR research is directed toward the resolution of scientific and regulatory issues that provide the basis for regulatory decisions.

An NCTR extramural Science Advisory Board, its subcommittees, and liaison members from each of the other FDA centers/ORA, actively provides guidance on the relevance and quality of these research efforts.

B.A. Schwetz, D.V.M., Ph.D.
Director, NCTR

SCIENCE ADVISORY BOARD

SCIENCE ADVISORY BOARD
TO THE
NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Function

One of the keys to maintaining a high quality research organization is the utilization of an outside body of experts, such as a Science Advisory Board (SAB), to periodically review the quality as well as the direction of the research. The NCTR SAB advises the Director in establishing, implementing and evaluating the research programs that assist the Commissioner of Food and Drug Administration (FDA) in fulfilling regulatory responsibilities. This additional review ensures that the research programs at NCTR are scientifically sound and pertinent to the FDA.

FY98 Accomplishments

During the year members of the SAB along with expert consultants conducted three site visits of the Centers' research programs in Biometry and Risk Assessment (October, 1997), Neurotoxicology (March, 1998), and Biochemical Toxicology (September, 1998). In May, the Board met as a committee of the whole.

At the full meeting of the Board, draft reports of the site visit reviews of the Biometry and Risk Assessment and Neurotoxicology programs with recommendations were presented and approved. The Board completed the approval, including a minority report of the review of the Center's Information Technology program. The Board was also given a progress report on the Estrogen Knowledge Project and recommended that it be reviewed again in the spring of 1999.

The site visit reports and the minutes of the SAB meeting can be accessed at http://intranet.nctr.fda.gov/.

The draft report of the site visit of the review of the Center's Biochemical Toxicology program along with reports on the Centers' Genetic Toxicology and Molecular Epidemiology programs to be site visited in October, 1998 and January, 1999 will be presented to the full Board in a meeting planned for late Winter, 1999.

The Board saw the retirements of Drs. Harold Davis of Amgen and Lily Young from Rutgers and the extension of the appointment for two years of the Board's current Chair, Dr. Marion Anders. The Center welcomes two new members to the Board, Drs. Catherine W. Donnelly and Stephen S. Hecht as it begins its twenty-sixth year of service to the Center and the FDA.

SCIENCE ADVISORY BOARD TO THE NCTR
Membership Roster

NAME/TITLE	AFFILIATION	TERM ENDS	EXPERTISE
Dr. Marion W. Anders Professor, Chairman, Dept. of Pharmacology	University of Rochester Rochester, NY	6/30/00	Veterinary Medicine, Biochem./Pharm.
Dr. Robert E. Anderson Professor Emeritus, West Virginia University	WV School of Environmental Education, Inc. Bridgeport, WV	6/30/00	Food Technology
Dr. William R. Bruce Professor, Departments of Medical Nutritional Science	University of Toronto Toronto, Ontario Canada	6/30/99	Medicine, Biophysics
Dr. Catherine W. Donnelly Associate Dean, College of Agriculture & Life Sciences	University of Vermont Burlington, VT	6/30/02	Microbiology/Food Science
Dr. Tómas R. Guilarte Professor, School of Hygiene and Public Health	Johns Hopkins University Baltimore, MD	6/30/99	Medical Physics, Zoology
Dr. Stephen S. Hecht Wallin Land Grant Professor of Cancer Prevention	University of Minnesota Cancer Center Minneapolis, MN	06/30/02	Chemistry
Dr. Joseph V. Rodricks Senior Vice President	ENVIRON International Corporation Arlington, VA	6/30/99	Toxicology/Risk Assessment
Dr. Marcy E. Rosenkrantz Director Information Institute	Air Force Research Lab Rome, NY	6/30/00	Computational Chemistry
Dr. Charles L. Wilkins Professor of Chemistry and Assoc. Dean Physical and Mathematical Science	University of California, Riverside Riverside, CA	6/30/00	Chemistry
Mr. Ronald F. Coene Executive Secretary Deputy Director, Washington Operations, NCTR	FDA/NCTR Rockville, MD	Ongoing	Research Administration

RESEARCH ACCOMPLISHMENTS AND PLANS

BIOCHEMICAL TOXICOLOGY

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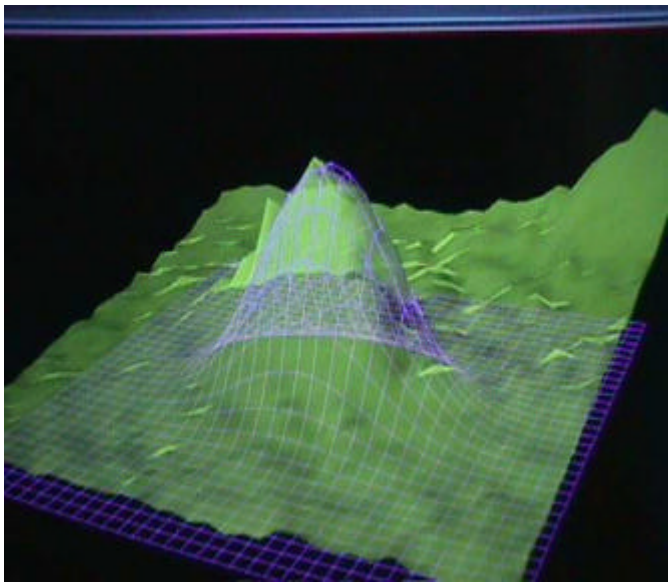

A 3-dimensional image of a DNA adduct detected using the ^{32}P-postlabeling assay

Introduction

The Division of Biochemical Toxicology conducts fundamental and applied research specifically designed to define the biological mechanisms of action underlying the toxicity of products either regulated by or of interest to the Food and Drug Administration (FDA). This research centers on the assessment of carcinogenic risk for specific chemicals and substances, and the introduction of new techniques to assess carcinogenic risk. The risk assessment research is firmly rooted in mechanistic studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in the subsequent carcinogenic risk assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic chemistry, cellular and molecular biology, immunology, nutrition, and pharmacology. It is supported by sound technical skills, the availability of state-of-the-art equipment, and internal and external collaborations and funding.

FY98 Accomplishments

During 1998, the Division conducted research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences National Toxicology Program (NIEHS/NTP). This emphasis reflects the fact that the NCTR has animal facilities that are rivaled by few, if any, research institutions. As such, the Center has the capability to conduct subchronic and chronic toxicological assessments in a rigorous manner to address the FDA's needs. While acknowledging the limitations of animal bioassays, these studies currently serve as the benchmark by which toxicological assessments are made by federal agencies, including the FDA. In addition to providing basic information on toxicological endpoints, such as cancer, these experiments form the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

The Division's NIEHS/NTP investigations have focused on the pediatric sedative chloral hydrate, in response to a request made by the Center for Drug Evaluation and Research (CDER), and the mycotoxin fumonisin B_1, which was nominated by the Center for Food Safety and Applied Nutrition (CFSAN). At the request of the Center for Veterinary Medicine (CVM), bioassays and mechanistic studies were initiated on malachite green, a therapeutic agent used in aquaculture. In response to a nomination from CFSAN, a chronic bioassay and associated mechanistic studies were started to elucidate the effects of ethanol on the carcinogenicity of urethane.

Recently much attention has been given to toxicities associated with endocrine disrupting chemicals. In response to this concern, a major new initiative was funded by the NIEHS/NTP. This program is focused on reproductive and carcinogenic endpoints of a number of chemicals including genistein, methoxychlor, nonylphenol, vinclozolin, and ethinyl estradiol. A major emphasis of the program is the elucidation of dose-response relationships over an extensive dose range. In addition to NIEHS/NTP-funded studies, other investigations within the Division have focused on endocrine disrupting chemicals. These include developing human cell lines that contain an estrogen-responsive reporter gene and also express phase I and phase 2 metabolic enzyme activities, investigating the metabolic activation pathways of the antiestrogen tamoxifen, and developing new methodologies for assessing metabolic differences in estrogen metabolism.

Another subject that has recently been funded by the NIEHS/NTP is in the area of phototoxicity. This investigation was initiated in response to a concern of CFSAN about the potential interaction between UV light and over-the-counter cosmetics containing α–hydroxy acids. It is anticipated that other potentially phototoxic compounds of interest to the FDA will be investigated in subsequent years.

Traditional chronic carcinogenicity bioassays are both very expensive and lengthy; thus, the development of alternative methods of assessing carcinogenic potential should be of great value. One approach that is currently being investigated is the neonatal mouse tumorigenicity assay. The advantages of this method are that only limited amounts of test material are required, a direct assessment is obtained as to whether or not the agent acts through a genotoxic mechanism, and less time is required to elicit a carcinogenic response. In collaboration with investigators at CDER, this alternative bioassay has been applied to benzodiazepines, antihistamines, lipid peroxidation products, estrogens, antiestrogens, peroxisome proliferators, and lipid peroxidation inducers.

An ongoing goal within the Division is to exploit both the immunogenicity and the antigenicity of toxicants, metabolites, and DNA adducts to develop and apply immunochemical methods combined with mass spectral techniques to address problems of regulatory concern including exposure, risk of toxicity, product screening, and mechanisms of toxicity. This technology has been applied to fumonisin B_1, fumonisin B_2, and fumonisin B_3; aromatic amine DNA adducts; nucleoside analogues of anti-HIV drugs; and ethane-type DNA adducts formed by urethane.

A major focus within the Division has been in the area of dietary folate and methyl deficiency in support of the FDA's decision on folate food fortification. Recent clinical and experimental data have linked nutritional folic acid status to both anticarcinogenic and procarcinogenic activities. Using an *in vivo* model of folate/methyl deficiency, Division investigators have developed a program to evaluate DNA damage and alterations in DNA methylation patterns during multistage hepatocarcinogenesis. Additional studies have focused on the relationships among folate status, polymorphisms in the methylene tetrahydrofolate reductase gene, and the incidence of Down Syndrome.

FY99 Plans

Method-/Agent-Driven Research

During 1999, final reports on the NTP-nominated chemicals fumonisin B_1 and chloral hydrate will be completed. The chronic bioassay on the interactions of urethane and ethanol will be completed, and a chronic bioassay with malachite green will be initiated. Range-finding and immunotoxicity studies will be completed on the endocrine disrupting chemicals genistein, methoxychlor, nonylphenol, vinclozolin, and ethinyl estradiol; a multigenerational bioassay will continue with genistein, and a multigenerational bioassay will be initiated with nonylphenol. In response to a request from CFSAN, protocols will be prepared to conduct a comprehensive toxicological assessment on α–hydroxy acids; chemo-exfoliants that are components of many skin care products.

Mechanistic studies will continue on fumonisin B_1, malachite green, urethane in the presence of alcohol, and endocrine disrupting chemicals. The fumonisin B_1 experiments will be centered on the isolation and characterization of ceramide synthase, a key enzyme involved in the toxicities of fumonisin B_1. In addition, an extensive short-term study will be completed with a number of fumonisin derivatives (fumonisin B_1, fumonisin B_2, fumonisin B_3, hydrolyzed fumonisin B_1, 2-hydroxypyridinyl fumonisin B_1, and carboxymethyl fumonisin B_1) to ascertain their contributions to the toxicities associated with *Fusarium*. Studies with malachite green will focus on the importance of DNA adduct formation in the suspected tumorigenicity of the dye and the metabolic pathways leading to these adducts. Experiments with urethane and ethanol will emphasize the DNA adducts formed by urethane and how these are affected by increasing concentrations of ethanol. Mechanistic studies with endocrine disrupting chemicals will include investigating the effects of dietary genistein on the growth of chemically induced mammary tumors, determining the distribution and metabolism of genistein, and characterizing the effects of endocrine disruptors on the metabolism of endogenous steroids and xenobiotics. In addition, experiments will continue to develop an *in vitro* human cell culture system to screen chemicals expected to have estrogenic or antiestrogenic activities. Further mechanistic studies will examine the DNA adducts formed by the mycotoxin riddelliine, a compound of interest to CFSAN that is undergoing an NTP-sponsored chronic bioassay.

Concept-Driven Research

The induction of mutations in critical target genes is regarded as an essential feature in the multi-step process of tumorigenesis and recent studies indicate that the ability of DNA adducts to induce mutations is quite dependent upon their three-dimensional structure within DNA. Experiments will continue to elucidate the conformation of specific DNA adducts and determine the effects these adducts have upon DNA structure. These conformational changes will then be related to the specific mutations induced by the DNA adducts in bacteria and animal models. A further goal is to elucidate the factors that dictate the sequence specificity for DNA adduct formation and removal.

Despite the acknowledged importance to human cancer prevention, the specific nutrient-gene interactions that promote malignant transformation are not yet understood. Chronic deficiencies in the methyl donors folate, methionine, and choline reproducibly induce liver tumors, reduce total folate levels, and alter distribution of folate derivatives. Since adequate folate availability is essential for maintenance of both deoxynucleotide DNA precursor pools and normal DNA methylation, experiments will continue to study the effect of folate deficiency on deoxynucleotide pool balance, base misincorporation, and cell death; and the progressive alterations in p53 gene methylation patterns and expression during liver tumor progression with chronic folate/methyl deficiency.

Down Syndrome, or trisomy 21, is a common birth defect that occurs in this country. The possibility that a gene-nutrient interactive effect may be involved in the etiology of Down Syndrome has not been investigated. A preliminary case-control study demonstrated that frequency of a 677C$\rightarrow$T polymorphism in the methylene tetrahydrofolate reductase (MTHFR) gene was increased in the mothers of Down Syndrome children. Because DNA hypomethylation has been associated with abnormal chromosomal segregation, this polymorphism could increase the risk of meiotic non-disjunction by promoting abnormal DNA methylation. Additional studies will focus on confirming this observation and exploring the mechanism by which this polymorphism increases the risk.

New Strategies for the Prediction of Toxicity

During the year, newborn mouse bioassays will be completed on specific classes of chemicals including estrogens, antiestrogens, peroxisome proliferators, and lipid peroxidation inducers. Bioassays will be continued on anti-HIV nucleoside analogues. These studies will provide critical information on the strengths and limitations of the newborn mouse bioassay by indicating the classes of chemicals to which it is sensitive.

Lipid peroxidation generates a number of products, such as malondialdehyde, formaldehyde, acetaldehyde, acrolein, and crotonaldehyde. Several of these lipid peroxidation products have been shown to be highly cytotoxic, genotoxic, mutagenic, and tumorigenic in rodents. In addition, they have been found to have the capability to bind covalently to cellular DNA, forming the endogenous DNA adducts that have been

detected in experimental animals and human tissues. Experiments will continue to develop sensitive and reliable analytical methodology for detecting and quantifying these endogenous DNA adducts in animal and human samples.

Major risk factors for women developing breast cancer are associated with increased estrogen exposure due to early menses, late menopause, or long-term estrogen replacement therapy. Estrogen carcinogenesis may result from hormonal effects, including promotion from receptor-mediated increases in cellular proliferation; metabolic activation of estrogens to reactive metabolites that bind to cellular macromolecules and act as chemical carcinogens; and/or estrogen or estrogen metabolite regulatory effects. Estrogen metabolites may modulate phase I and phase II enzymes that bioactivate and detoxify breast carcinogens. Further, cytokines have emerged as important regulators of estrogen synthesis in breast tissues. In order to determine the relative importance of these factors, additional biomarkers are needed that can be applied to noninvasively obtained human samples. With this goal in mind, a new project will be initiated to use immunochemical and mass spectral techniques to measure specific estrogen metabolites.

Significance to the FDA

The FDA is entrusted with the responsibility of ensuring the safety of foods, drugs, biologics, medical devices, and cosmetics. The identification of carcinogens has depended classically upon two approaches, epidemiological studies and chronic animal bioassays, each of which has its own strengths and weaknesses. The development of new techniques to assess carcinogenic risk provides the basis for alternative methods of assessing carcinogenic potential that can augment, or perhaps even replace, the need for expensive chronic bioassays. The FDA benefits from these studies by having improved pre-clinical assessments that allow extrapolations across species and lead to more scientifically sound risk decisions for the public. The outcome of the Division's research is an integrated suite of technology for use in guiding pre-clinical assessments of compounds regulated by the FDA.

BIOMETRY AND RISK ASSESSMENT

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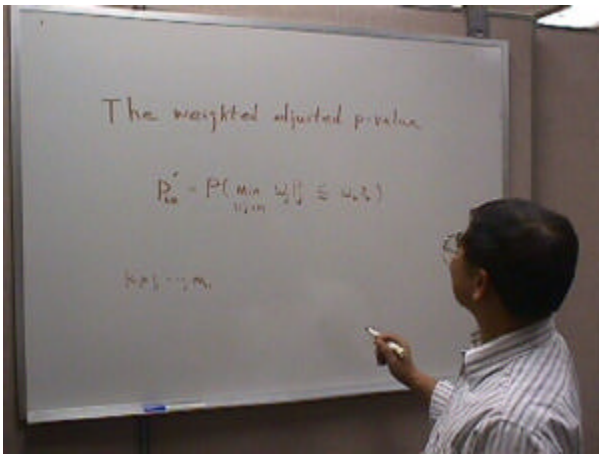

Dr. Jim Chen working on a new biometrical method for hazard identification.

Introduction

The regulation of toxic substances in foods, drugs, biologics, cosmetics, animal drugs, and medical devices requires an engagement in risk assessment. Risk assessment is a process for determining the extent of human health hazard as a function of the conditions of exposure to toxic substances. It may involve the derivation of numerical estimates of risk corresponding to specific exposure levels, or it may simply involve determining that such exposure levels are sufficiently low to pose a negligible risk to those exposed. The daily dose rate, the route of exposure, the age at exposure, and the duration of exposure are all factors that can influence risk.

The mission of the Division of Biometry and Risk Assessment is to conduct research to address FDA's regulatory need for new and improved methods of risk assessment; to assess the uncertainty associated with current approaches; and, to develop and apply new methods for the assessment of human exposure, susceptibility, and risk.

In most cases, assumptions must be made in order to extrapolate results observed at high doses in animal experiments to doses below the experimental range, to extrapolate across different routes and durations of exposure, and to translate animal risks (exposure levels) into human risks (exposure levels). Consequently, the uncertainty involved in estimating risks and in setting acceptable exposure levels can be substantial. Research is being conducted in the Division of Biometry and Risk Assessment to properly account for such uncertainty in the risk assessment process and, ultimately, to reduce this uncertainty. This research is directed toward the derivation of new methods as well as the assessment of current methods.

The research is relevant to FDA's strategic goals of improving the pre-market review process and of establishing strong post-market assurance standards. The research spans all of NCTR's strategic research goals: the development of knowledge bases; the development of new strategies for the prediction of toxicity; and the conduct of method-, agent-, and concept-driven research.

FY98 Accomplishments

Scientists in the Division of Biometry and Risk Assessment had 15 first-authored research papers accepted for publication, and co-authored an additional 10 papers. Major research accomplishments under each of NCTR's strategic research goals were as follows:

Development of Knowledge Bases

Research was continued on the feasibility of developing a knowledge base for predicting the outcome of the two-year cancer bioassay using results of short-term tests (X70048). The approach developed in FY97 for using a logistic regression model to translate short-term results into long-term predictions was refined. A manuscript demonstrating that it is possible to identify chemicals with very high likelihood of giving a positive result in the bioassay and those with very low likelihood of giving a positive result has been accepted for publication. For such chemicals, it might be possible to eliminate the conduct of a two-year bioassay. If so, the approval process of certain human and animal drugs, food additives, and medical devices could be accelerated.

New Strategies for the Prediction of Toxicity

A joint project with scientists in CFSAN's Office of Pre-Market Approval to investigate the feasibility of expanding the Threshold of Regulation for indirect food additives from food-contact articles to include direct-flavor additives was begun (S00174). A preliminary analysis has been completed, with promising results. In a related activity, participation in the re-write of the Flavor and Extract Manufacturers' Association (FEMA) decision-tree strategy for classifying flavors with respect to their toxic potential was begun.

A manuscript describing a unified approach to safety assessment for both carcinogenic and noncarcinogenic effects was accepted for publication (S00174). The approach is directed toward restricting the use of mathematical models to the range of observed data, and using a consistent method of extrapolating to acceptable levels of exposure, which de-emphasizes numerical estimates of risk. A second manuscript has been submitted.

Research was conducted on the use of a pharmacokinetic database for extrapolation from animals to humans (P00393). The problem of lack of standardization of pharmacokinetic analyses and reporting was addressed. A consistent, multi-species pharmacokinetic database for interspecies extrapolation was designed. Work was begun on populating the database with pharmacokinetic data on dexamethasone and methyl mercury. Two manuscripts were accepted for publication in FY98.

A new protocol was developed for conducting research on linking physiologically based pharmacokinetic (pbpk) models to biologically based dose-response (bbdr) models in order to improve estimates of cancer risk (E07030.01). This research will facilitate the incorporation of time-dependent measures of target-tissue dose into stochastic models of carcinogenesis, thereby refining predictions of risk.

Method-Driven Research

Research was continued on the development of statistical methods for attributing cause of death in animal tumorigenicity studies (E06896.01). A statistical method was developed to partition the group of animals that die with tumors during the course of an experiment into those that die because of the tumor and those that die from a competing risk. Monte Carlo simulation results indicate that the numbers of fatal and incidental tumors imputed using this method are within reasonable error limits. The work resulted in four manuscripts.

Work continued on research to extend the two-stage, clonal-expansion model for carcinogenesis (E6908.01). During FY98 a comprehensive tool for stochastic carcinogenesis modeling was produced in *Mathematica.* The package is named CarcinoMod. In addition, two manuscripts were accepted for publication.

Work continued on characterization of the joint action of toxicants, and on risk estimation for mixtures of chemicals (E06984.01). One manuscript was submitted during FY98. Participation in the cross-agency Mixed Exposures Risk Group (MERG), spearheaded by EPA, was begun.

Work continued on a protocol on statistical tests involving multiple end points (E07009.01). Two manuscripts were accepted for publication, one on a new procedure for adjusting p-values of individual comparisons in order to control the family-wise error rate and the other on the simultaneous analysis of multiple tumor types. Monte Carlo simulation results have shown that the procedures perform well under a variety of conditions.

A protocol was developed to investigate the use of a mixture of normal densities for enzyme variant classification based on the induction of CYP1A2 (E07037.01). Identifying genetic variants within the human population with respect to key enzymes in activation or detoxification pathways is important for evaluating relative disease risks for groups of people of varying susceptibility. A manuscript on kernel density estimation for polymorphic populations was submitted for publication in FY98.

Work continued on the development of mathematical models of human embryonic/fetal growth from implantation to birth (P00393). Good fits to growth data were obtained by nonlinear regression through numerical integration of an extended Gompertz model. One manuscript was submitted during FY98.

A chapter on risk assessment was written for a document being prepared by the National Research Council's Committee on Toxicology to provide guidance to NASA in developing spacecraft maximum allowable limits for water contaminants (S00032). The chapter espouses several new approaches to risk assessment, including the use of benchmark doses and a new way to combine uncertainty factors.

A chapter on the statistical analysis of data from reproductive/developmental toxicity studies was written for the Encyclopedia of Biopharmaceutical Sciences (S00032). The chapter discusses historical approaches along with more modern approaches, a number of which were developed at NCTR.

Research continued on estimating risks and calculating benchmark doses for nonquantal toxicity data, including developmental neurotoxicity data, data involving mixtures of populations, and change-point dose-response data (S00116). Two manuscripts were submitted during FY98.

Agent-Driven Research

The dosimetry of dexamethasone (DEX) was characterized by various pharmacokinetic parameters, and their impact on developmental toxicity endpoints was evaluated (E06638.12). One manuscript was submitted for publication and a final report was submitted.

A number of clinical chemistry and hematology parameters were measured and characterized in the pregnant and lactating laboratory rat at multiple time points in order to establish normal blood parameters for the pregnant rat (E06957.11). Such values are noticeably absent from the open literature. A manuscript was submitted characterizing 15 clinical chemistry parameters and eight hematology parameters. A final report was submitted.

A protocol was developed and approved for studying mortality among atomic bomb survivors who were exposed *in utero* or as young children (E07029.01). This work is being done in collaboration with scientists at the Radiation Effects Research Foundation (RERF) in Japan.

Concept-Driven Research

A major effort was expended to conduct a series of statistical analyses to evaluate body-weight data, survival data and tumorigenicity data from the studies carried out under the Project on Caloric Restriction (E00501-E00509). The development of a series of manuscripts to report these results was begun. During FY98 three manuscripts dealing with the implications of caloric restriction for risk assessment were accepted for publication, including an expository paper on the use of nutritionally adequate dietary restriction to control body weights in rodent chronic studies for the purpose of reducing body-weight-related variability in survival and tumor incidences.

FY98 Interactions with FDA Centers

- Collaborated with scientists at CFSAN on standardized growth models of experimental animals for reconstruction of whole-animal pharmacokinetics.

- Collaborated with scientists at CFSAN to investigate expanding the Threshold of Regulation to direct flavor additives (see above for fuller explanation).

- Collaborated with scientists at CDER on the analysis of dissolution curves.

- Collaborated with scientists at CDER on a guidance document for pre-clinical evaluations of gerontotherapeutics.

- One staff scientist served a 120-day detail at CDER to participate in the pharmacology review of a gerontotherapeutic IND.

FY98 Center-wide Support

- Provided statistical consultation on a variety of experiments in support of the divisions of Biochemical Toxicology, Genetic and Reproductive Toxicology, and Molecular Epidemiology.

- Provided oversight to the statistical-analysis support group and the experimental-liaison support group under the center-wide information-management contract, and reviewed all protocols for automated data processing requirements.

- Provided guidance to the Institutional Animal Care and Use Committee regarding statistical justifications of animal requirements for in-house experiments.

Other FY98 Accomplishments

Division scientists, through invited presentations at national and international meetings, workshops, universities, and other government agencies have broadened the impact of NCTR's research efforts to improve the risk assessment process. They have distinguished themselves as conference organizers, committee members, program reviewers, and associate editors of peer-reviewed scientific journals.

FY99 Goals

1. To develop statistical testing methods and predictive systems for identifying potential health hazards associated with toxic substances;

2. To develop biometrical methods for estimating risks associated with toxic substances to enable setting exposure levels that correctly reflect underlying uncertainties;

3. To develop mathematical models for better representation of internal exposure levels and biological mechanisms in order to reduce uncertainty in estimates of risk;

4. To provide statistical expertise to NCTR scientists on the design, conduct and analysis of research studies to evaluate the toxicity of regulated products;

5. To assist other FDA centers in conducting risk assessments for the regulation of specific products and in investigating risk-assessment issues; and

To participate in interagency risk-assessment activities to maintain knowledge of the state-of-the-art and to promote the improvement and unification of risk-assessment practices across agencies.

FY99 Plans

All ongoing projects which have not been completed will continue into FY99. In addition, some current projects will be expanded and several new projects will be initiated, as follows:

New Strategies for the Prediction of Toxicity

A Monte Carlo simulation study for the collaborative project with CDER on shortened bioassays for assessing the toxicity of drugs (E06902.01) will be completed. Results will be evaluated with respect to the statistical implications of such shortened bioassays.

Research under the protocol for rodent 3-dimensional reconstruction and animation (E06953.01) will be completed. A newly acquired motorized stage will enable microscopic slide reading of the resolution needed for 3-D imaging.

Work will continue on the joint project with CFSAN to investigate the feasibility of expanding the Threshold of Regulation for indirect food additives to include direct flavor additives (S00174). This approach has the potential to provide a defendable basis for a determination that toxicity testing is needed or not.

Participation will continue in the rewriting of the document describing the Flavor and Extract Manufacturers' Association (FEMA) decision-tree strategy for classifying flavors with respect to their toxic potential (S00174).

Research will continue on the use of a pharmacokinetic database for extrapolation from animals to humans (P00393). The database will continue to be populated initially with pharmacokinetic data on dexamethasone and methyl mercury. The comparative performance of traditional pk models to the more recent pbpk models for interspecies extrapolation will be evaluated.

A new protocol will be implemented for conducting research on linking physiologically based pharmacokinetic (pbpk) models to biologically based dose-response (bbdr) models in order to improve estimates of cancer risk (E07030.01). The impact of allowing biological parameters of integrated pbpk-bbdr models to vary among individuals will be explored.

A new protocol to conduct research on dose-response models for microbial risk assessment will be finalized (E07045.01). The objectives of the research are to develop improved models for estimating probabilities of infection and disease, and to develop methods for incorporating model uncertainty into microbial risk assessment.

Method-Driven Research

The statistical method developed in FY98 for imputing the numbers of fatal and incidental tumors (E6896.01) will be applied to modify the cause-of-death test of the International Agency for Research on Cancer (IARC). This test is widely used to evaluate preclinical data in pharmaceutical development. Successful modification of the IARC test would eliminate the need for pathologists to assign a cause of death to each animal in a bioassay.

Work will continue on extending the Moolgavkar-Venzon-Knudson (MVK) model for carcinogenesis (E6908.01). In particular, an extended MVK model developed under this protocol will be explored in terms of whether u-shaped dose-response curves can occur even for genotoxic carcinogens with nonzero background risk. If so, this finding could have significant impact on standard assumptions regarding low-dose linearity for cancer dose-response models.

Work will be completed in FY99 on developing a proportion-concentration model for characterization of the joint action of toxicants, and on comparing methods for risk estimation for mixtures of chemicals (E06984.01). This work will be complemented by participation in the cross-agency Mixed Exposures Risk Group (MERG).

Research will continue on statistical tests involving multiple endpoints (E07009.01). Procedures for the simultaneous analysis of multiple tumor sites and for the adjustment of individual p-values in rodent studies for carcinogenicity will be compared with respect to power.

Work will begin on the protocol to investigate the use of a mixture of normal densities for enzyme variant classification based on the induction of CYP1A2 (E07037.01). Characterizing human subjects with respect to their differential enzyme induction is a key to studying interactions of various risk factors in molecular epidemiology.

A protocol will be written for developing a simultaneous exact method for assessing equivalence trials (E99999). This research is intended to respond to the current intense interest within CDER and in the regulated pharmaceutical industry in the development and use of statistical methods for assessing bioequivalence.

Research will continue on estimating risks and calculating benchmark doses for nonquantal toxicity data, including developmental neurotoxicity data, data involving mixtures of populations, and change-point dose-response data (S00116).

Agent-Driven Research

Collaborative research with scientists at the Radiation Effects Research Foundation in Japan will continue under the protocol for studying mortality among atomic bomb survivors who were exposed *in utero* or as young children (E07029.01).

Concept-Driven Research

Statistical analyses will be completed on the series of experiments conducted under the Project on Caloric Restriction (E00501-E00509). The remaining between-study comparisons will be made and a series of manuscripts will be submitted for publication.

Significance to the FDA

The Division of Biometry and Risk Assessment is a focal point within FDA for research in the area of Health Risk Assessment. Human health risk estimates impact on the regulation of exposure to toxic substances, which affect the health, and economy of the U.S. population. The Division of Biometry and Risk Assessment has the mission of identifying uncertainties in the risk assessment process, and developing risk estimation techniques that either appropriately account for or reduce these uncertainties, in order to improve the regulation of natural or synthetic toxic substances occurring in foods, drugs, biologics, cosmetics, animal drugs and medical devices. Continued significance to the FDA is fostered through interactions with individuals and committees at other FDA centers that are involved in evaluations of risk for the regulation of specific products.

CHEMISTRY

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Introduction

Ms. Willie May Cooper conducting lipid analysis on feed samples in support of chemicals nominated by the FDA for National Toxicology Program studies.

The mission of the Division of Chemistry is to provide analytical chemistry support in the areas of methods development, mass spectrometry and nuclear magnetic resonance, along with maintaining an active research program to develop and explore new technologies in response to the needs of the FDA. Enforcement of regulations requires sound, reliable and validated analytical procedures as the basis of implementing regulations governing adulterants, contaminants, additives, as well as the composition and efficacies of FDA-regulated products. Rulings will not withstand legal scrutiny without validated analytical procedures as their basis. In recognition of this, NCTR is applying its collective expertise and equipment base in a methods development program tailored to FDA goals. Methods are being developed and validated in support of the FDA-nominated compounds to the National Toxicology Program (NTP) and staff chemists are supporting biologists, toxicologists, immunologists in their efforts to characterize test agents and to improve toxicity assessments. In an effort to improve the nation's food supply through the Food Safety Initiative and using similar technology to address the threat of bioterrorism, scientists within the division are developing methods to identify deleterious bacteria using mass spectrometry. Research on development of methods and devices for efficient determination of food and seafood quality is also being conducted. The program has a strong commitment to development of analytical methods that are prerequisites for determination of test chemical purity, stability and homogeneity in dosage forms and dosage certifications for chemicals scheduled for toxicological evaluation under the NTP. These include fumonisin B_1, chloral hydrate, urethane, leuco-malachite green, malachite green, genistein, vinclozolin, ethinylestradiol, nonylphenol, and methoxychlor.

The development of analytical methods to support FDA's regulatory and enforcement actions extend beyond the scope of traditional analytical chemistry. Analytical chemists provide evidence regarding the bioactive/bioavailable forms of regulated compounds, as well as evidence regarding mechanisms of action, individual susceptibility, and potential avenues to minimize the risks associated with exposure to toxicants. Research in analytical chemistry, mass spectrometry and nuclear magnetic resonance provides the

means to create, develop, or modify instruments needed to measure the levels of analytes of importance to FDA.

The measurement and confirmation of constituents of interest in food, drug and cosmetic products regulated by the FDA are necessary to minimize possible human exposure (or effects of exposure) to potentially harmful chemicals. New analytical capabilities may be required to deal with hazards that may arise in the future. The range of projects in this program is indicative of the diverse nature of the regulatory responsibilities of the Agency. Projects are selected based on Agency priorities and programmatic expertise.

FY98 Accomplishments

Analytical Chemistry

During FY98, the Division developed methods of analysis to measure numerous compounds scheduled for study under the NTP. One specific study required detection sensitivity down to the 1 part per billion (ppb) level of endocrine disrupter ethinyl estradiol in a variety of matrices in support of multigenerational studies conducted at NCTR in partnership with NTP. Over fifty percent (50%) of the Division's efforts have been devoted to developing or adapting analytical techniques to confirm purity, to measure dosages in feed and to insure that purchased diet was free of trace levels of contaminants. In support of studies conducted by NCTR research divisions, the Division of Chemistry conducted dose certification, stability and diet homogeneity studies on: chloral hydrate, genistein, methoxychlor, nonylphenol, urethane, vinclozolin, and ethinylestradiol.

As required by Good Laboratory Practices, the Division fulfilled the function of chemical custodian, which required that all uses of the test compounds were documented and certified measurements were used to prepare all dosed feed. Chemical summary reports were prepared or are in preparation for all compounds under test.

Under the category of Analytical Chemistry, studies continued to explore the health implications of botanical dietary supplements. Several papers were published and collaborative projects have been submitted for outside funding to continue this potentially critical FDA work.

The Division of Chemistry has worked to develop a number of multi-residue methods, primarily involving antibiotics. Recently, work was presented on the determination of amoxicillin residues in beef, pork, chicken and tilapia by liquid chromatography using fluorescence detection. Multi-residue methods were also developed to determine and confirm sulfonamides in edible tissues of aquaculture products. This included the extraction and HPLC separation of 14 aquaculture-related sulfonamides.

A joint project between the Division of Microbiology and the Division of Chemistry resulted in a publication entitled “Influence of selected physical factors on the biodegradation of acrylamide by immobilized cells of *Rhodococcus sp.*”

Methods for analysis and confirmation of erythromycin A residues in tissue samples from terrestrial and aquatic farmed animals, by HPLC, were also developed. Liquid chromatography-electrochemical detector (LC-ECD) methods for determination of erythromycin A in chicken liver were evaluated and LC/MS methods for confirmation of this antibiotic in chicken liver are being developed.

Scientists within the Division of Chemistry have conducted studies in partnership with the NTP and other center scientists to address the induction of tumors in rodents maintained on an idealized weight curve. This is a carryover project from the Project on Caloric Restriction and adds to the knowledge base associated with the tumorigenicity of chloral hydrate. Using weight control procedures greatly reduces the variability in such end points as liver-to-body-weight ratio and cytochrome P450 induction. Preliminary results from the pathological analysis show that in the dietary controlled mice there was a dose-dependent increase in liver tumors due to chloral hydrate treatment. This was less apparent in the *ad libitum*-fed groups. Chloral hydrate-induced liver tumor formation appears to correlate with induction of enzyme markers for peroxisome proliferation suggesting a causal relationship. A microtechnique for the GC analysis of chloral hydrate metabolites in blood plasma and a humane technique for collecting serial blood samples by tail-clip from mice were developed and allowed the pharmacokinetic measurement of chloral hydrate and its metabolites by serial sampling of individual mice.

Analytical chemists in the Division are turning their attention to the measurement of endocrine disruptors in plasma. Besides developing the methods for analyzing genistein in feed, methods have been developed for the measurement of genistein, daidzein and their glucuronide metabolites in human and rat plasma at the one micromolar level.

A patent application for a color-based fish freshness indicator test-strip was sent to FDA and subsequently submitted to the U.S. Patent Office that gives a measure of the volatile bases associated with seafood decomposition. The test strip directly senses volatile bases resulting in a color change in the test-strip even at low temperatures. The method can also be applied to beef, pork or poultry.

Mass Spectrometry

The mass spectrometry laboratory has both a support and research component. In the support area in FY98, over 700 samples have been analyzed for five separate divisions at the Center. Collaborative work has also been initiated with the University of Arkansas for Medical Sciences and samples have been run in support work of this collaboration.

The mass spectrometry laboratory continues to work closely with the analytical chemistry group to develop very sensitive methods for the detection of endocrine disruptor compounds. One example of this close coordination was the methods development for ethinylestradiol. Working together, chemists developed a separation technique and the mass spectrometry team coupled it to a selected ion-monitoring mass spectrometry detection method for quantitation down to the 1 ppb level. Efforts are continuing to develop methods to measure this compound at the 100 ppt level using similar techniques.

In the research area, scientists within the Division are developing new technologies to characterize bacteria using mass spectrometry and develop patentable LC/MS interfaces. Analysis of a large number of isolated HPLC fractions has indicated the identity of two biomarkers observed in the mass spectra from whole cells of *E.coli* and *Shigella flexneri*. Both of these peaks are in fact proteins, corresponding to major portions of *E.coli* HdeA and HdeB. In each case, the first ten amino acids from the fraction collected from *Shigella* corresponded to the known *E.coli* proteins. These proteins are important because they are attributed to the sigma-s-dependent genes, encoded by rpoS, associated with an acid resistant phenotype. This work demonstrates that matrix-assisted laser desorption ionization (MALDI)-detected peaks from whole bacteria can be used as biomarkers to monitor this specific type of acid resistance in bacteria.

Work done under a Cooperative Research and Development Agreement (CRADA) Scientific Instruments Service continues to investigate the commercialization of an universal HPLC interface under development at NCTR. This interface allows many gas-phase detectors to be directly connected to liquid separation devices such as HPLCs.

Nuclear Magnetic Resonance Research and Support

With the addition of staff in FY98, a renewed effort is being mounted to continue nuclear magnetic resonance (NMR) support for mechanistic studies and metabolite identification. Research efforts are underway to use ^{13}C chemical shifts, mass spectrometry data, UV spectral data, molecular weight and solubility coupled with a known estrogenic binding activity to enhance quantitative structural activity relationship (QSAR) models. These additional data elements will serve to expand the structural database thus improving the estrogenic predictability of unknown compounds submitted for regulatory review.

Another study that may have significant effects on FDA's regulatory process is a joint effort between the Center for Drug Evaluation and Research and NCTR. Scientists at both centers are evaluating the role NMR methodology will have in improving the quality of some pharmaceuticals and increase the speed and lower the cost of drug development.

Surveillance Activities

The Division of Chemistry is also responsible for monitoring the feed, bedding and water quality at the Center. All feed and bedding samples are monitored for compliance to specification and potable water is frequently monitored for allowable levels of trace metals. Wastewater is also monitored to insure that Arkansas Pollution Control & Ecology permit criteria are met for quality and allowable levels of dissolved metals.

FY99 Goals

Analytical Chemistry

Continued support of the NTP efforts at NCTR is a critical part of the Division's future goals. In negotiations with the other centers' study directors, the Division of Chemistry will begin developing analytical methods for measuring endocrine disruptors in plasma and for characterization and quantitation of metabolites in biological matrices.

Future areas of interest to FDA are botanical-based dietary supplements. In order to anticipate the regulatory problem that may arise from this class of chemicals, studies are underway to evaluate the antimicrobial characteristic of herbal products and support efforts to help evaluate the developmental toxicity and potential carcinogenicity of these products.

Efforts continue in the field of food quality detection. Scientists within the Division are building on their success at monitoring the freshness of seafood, to develop products that will be capable of monitoring the freshness of all classes of food products.

Work will continue with CVM to validate their methods for amoxicillin and lincomycin in salmon. The determinative step in the method for sulfonamides in fish tissues will be validated in additional species and supercritical fluid extraction (SFE) will be investigated as a potential extraction method. Methods for analysis of erythromycin A residues in catfish and salmon tissue samples will also be validated.

Mass Spectrometry

Work will continue with the collaborative development of methods between mass spectrometry and analytical chemistry groups to support the NTP studies and to implement or conduct chemical characterization and identification studies.

In support of the FDA bioterrorism and food safety initiatives, mass spectrometry staff will further define the parameters necessary to identify resistant determinants in bacterial species by mass spectroscopy. It is hoped that by identifying the causative proteins in fractions from antibiotic- and non-antibiotic-resistant bacteria that an assay procedure can be developed based on antibiotic-resistance profiling to quickly and accurately measure deleterious bacteria in the field at very low levels of contamination.

NMR Research and Support

A continual collaborative interaction will be maintained with the Estrogen Knowledge Base research group to provide structural data for inclusion in database structure.

Also, research will be initiated into using one-dimensional and two-dimensional NMR techniques to determine the structure of DNA-adducted oligonucleotides. This will help to elucidate the structure of potentially toxic drugs that may bind to DNA.

The drug purity work will continue and publications will be drafted to apply this technique to the drug review process.

Significance to the FDA

The development and validation of relevant analytical methods will enable Center scientists and the Agency to perform analyses of food, drug and cosmetic products for constituents that the FDA has responsibility for regulating in order to make rapid decisions on the disposition of the products requiring action.

The development of analytical chemistry methods will allow the extension of analytical chemistry techniques to the analysis of complex biomolecules using analytical approaches quite different from those typically developed for more "traditional" molecules such as those produced by organic synthesis methods. In some instances, analytical chemistry will provide evidence regarding the bioactivity, bioavailable, or bio-altered form of a regulated compound not present in tissue residues as the expected parent compound.

The development of specialized instrumentation or novel applications will allow measurements that were not previously feasible to be accomplished, or may significantly reduce the cost of current analyses.

GENETIC AND REPRODUCTIVE TOXICOLOGY

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Research support scientist, Angela Harris, examines an autoradiogram of a gel indicating differential gene expression of toxin exposed human hepatocytes

GENETIC TOXICOLOGY LABORATORY

Introduction

The FDA requires that petitioners provide data evaluating the potential genotoxic activity of food additives, and human and animal drugs and biological therapies as part of the product approval process. Thus, the identification and quantitative measurement of the potency of suspected mutagens and carcinogens are essential to the regulatory function of the FDA. Regulatory decisions require not only the identification of potentially hazardous genotoxic and putative nongenotoxic chemicals, but also an understanding of their mode of action. The Genetic Toxicology Laboratory conducts fundamental research designed to define the pathways from initial DNA damage to mutation. Although this research is focused on the manner in which mutational events are related to the carcinogenesis process, an increased understanding of how mutations are transmitted to future generations can also be an outcome. Research within the Genetic Toxicology Laboratory centers on the development and validation of new techniques and methods by which to assess genetic risk within a framework of an increased understanding of the mutational process.

The approach of this laboratory has been to focus on the development and validation of *in vivo* systems to measure spontaneous and induced somatic mutations at multiple loci. In addition, the laboratory has made substantial progress in detecting the broad spectrum of genetic events that occur during the carcinogenesis process. Knowledge of systems with an increased capability to detect genetic damage, accompanied by studies designed to increase understanding of mutational mechanisms, will provide the review process with the most current information on which to base regulatory decisions. In order to maintain awareness for the regulatory process, new initiatives designed to develop more reliable and sensitive transgenic mutation detection systems are in development. In addition, a mechanistic study that addresses the effect of carcinogen-induced alterations on gene expression on the mutational process has recently been implemented.

FY98 Accomplishments and FY99 Plans

Experiments currently being conducted in the *in vitro* component of the Genetic Toxicology Laboratory either address immediate problems facing the FDA or are using model compounds to develop, characterize and validate new methods that can be applied to FDA-related problems. Examples of the former are recent efforts to determine whether several human drugs known to be genotoxic in model systems and carcinogenic in rodents, were genotoxic in transgenic and nontransgenic human lymphoblastoid cells. The drugs evaluated were tamoxifen, phenolphthalein and chloral hydrate and the genetic endpoints measured included micronuclei and mutations at the thymidine kinase (*Tk)* and hypoxanthine-guanine phosphoribosyltransferase (*hprt*) loci. The results indicated that all three induced micronuclei while only chloral hydrate induced mutations and predominately at the *Tk* locus. The phenolphthalein work was recently accepted for publication. A second intense area of inquiry using human lymphoblastoid cells deals with the role of programmed cell death in the recovery of mutants. In cultures of AHH-1 $Tk^{+/-}$ cells exposed to a series of clastogens, concentration-dependent increases in the percentage of apoptotic cells were accompanied by increases in the mutant fraction at the *hprt* locus or *Tk* locus of surviving cells. The recovery of cells with DNA damage indicated that apoptosis was not fully efficient in the destruction of damaged cells. These observations led to a series of experiments to assess the *p53* functional status of the cells as well as extended studies to determine if the functional status of the *p53* gene affected the recovery of mutant clones by modulating either the rate of apoptotic cell death or through an effect on cell proliferation. These studies were carried out using the phytoestrogen, genistein, a compound of interest to the FDA and other regulatory agencies.

In vivo experiments to understand the role of somatic mutation in the carcinogenesis process include continued characterization of the suitability of the X-linked *hprt* endogenous gene as a reporter gene for cancer genes; characterization and validation of the *lacI* and *MX174* transgenes as reliable and sensitive reporter genes for cancer genes; and utility of a $Tk^{+/-}$ transgenic mouse to detect genetic events associated with the carcinogenesis process and comparison of the sensitivity of this autosomal gene with the *hprt* gene. Thirteen mutagenic carcinogens targeting a variety of tissues were evaluated in the rat lymphocyte *hprt* assay in the laboratory. Of these, 12 induced mutations at the *hprt* locus indicating the usefulness of this gene and tissue as reporters for cancer genes. Other accomplishments include: 1) development of polymerase chain reaction/denaturing gradient gel electrophoresis DNA (PCR/DGGE/DNA) sequencing analysis for rat *hprt* exons 2, 3, and 8; 2) development of reverse transcriptase - polymerase chain reaction (RT-PCR) cDNA-sequencing analysis of point mutations in rat and mouse *hprt* mutants; and 3) development of a deletion screen for rat *hprt* using multiplex PCR. These molecular tools were used to analyze spontaneous, N-ethyl-N-nitrosourea (ENU), dimethylbenz(a)anthracene (DMBA), 1,6-dinitropyrene, 2-acetylaminoflorine (2-AAF), N-hydroxy-2-AAF and thiotepa- induced mutant sets.

Comparison of the mutability of a transgene to an endogenous gene is extremely important to assess accurately the relevance of transgenic systems and to determine their potential to complement or replace present rodent genotoxicity assays. Validation of the Big BlueRat transgenic model was accomplished by comparing mutant frequency between *lacI* and *hprt* in splenic lymphocytes following DMBA exposure; comparison of DMBA-induced *lacI* mutations in target and non-target tissues; comparison of DMBA-induced mutation spectra in *lacI* and *hprt* from lymphocytes of Sprague-Dawley F344 and Big Blue Rats; comparison of DMBA-induced DNA adducts in target and non-target tissues of Big Blue Rat and their correlation with *lacI* mutation frequencies; and *cII* mutation assay and comparison of mutation frequencies between *lacI* and *cII* in target and non-target tissues. A second transgenic model under investigation is the MX174 *am3 in vivo* mutagenesis model. This model has a number of potential advantages over the presently available 8-based models including a relatively low spontaneous mutation frequency making detection of weak mutagens possible. Studies to date include: 1) determination of the sensitivity of assay by comparison of ENU-induced mutant frequency in *am3* with lymphocyte *hprt* frequency; 2) development of a cell line from *am3* mice for use in detecting chemically-induced mutation and further characterization of the system; and 3) partial development of a forward assay using the *am3* transgene. A third transgenic model being developed and characterized is the $Tk^{+/-}$ mouse generated via homologous recombination using mouse embryonic stem cells. This model was created in order to detect mutagens exhibiting a wide spectrum of genetic events including recombination, deletion, loss of heterozygosity and single gene mutation. Accomplishments thus far include: 1) derivation of the $Tk^{+/-}$ mouse; 2) establishment of conditions for quantifying *Tk* lymphocyte mutants; and 3) measurement of spontaneous, ENU- and radiation-induced mutant frequencies.

A natural outgrowth of the laboratories desire to detect and characterize *in vivo* somatic mutation is the development of genotypic selection techniques. Genotypic selection refers to the detection of mutation solely upon the change in the DNA sequence that is itself the mutation. This technology requires analysis of the DNA from a target tissue in the absence of the use of intermediary steps such as cloning of endogenous genes in dividing tissues or amplification of a transgene via growth in a bacterial host. Accomplishments to date include:1) development of a MutEx assay for measuring CAA→AAA mouse H-*ras* mutation; 2) development of an Allele-Specific Competitive Blocker (ACB) polymerase chain reaction (PCR) assay for measurement of mutation at a frequency of 1×10^{-5}; and 3) combination of MutEx with ACB-PCR for measurement of mutation at a frequency of 1×10^{-7}. Recently a protocol describing plans for validation of MutEx/ACB-PCR for risk assessment by measuring spontaneous and induced H-*ras* mutation frequencies in various strains of tumor-prone and -resistant mice has been approved.

New initiatives are directed toward development of more reliable and sensitive transgenic targets and understanding how chemical carcinogens modulate gene expression thereby contributing to the fixation of mutations. The new transgenic targets include the use of fluorescent markers and microsatellite instability for the detection of

mutations. The gene expression initiative proposes to identify and analyze aflatoxin B_1 responsive genes in primary cultured human hepatocytes using differential display PCR and differential hybridization of a high-density filter array.

Summary

The work described is directed toward understanding the mechanism(s) of somatic mutation induction in model non-transgenic and transgenic *in vivo* systems and validating their usefulness for predicting the carcinogenicity of chemicals of interest to the FDA and the NCTR in order to provide the product review process with the most current information on which to base regulatory decisions. The methodologies range from quantification of mutation for genetic risk assessment to mutational spectra analysis for reliable and accurate cross-species comparison. The desire to develop highly sensitive techniques to detect mutagens at low doses as well as mutagens whose primary mode of action does not include direct DNA adduction is a primary motivation for the direction of the research. Included in the research activities are attempts to determine how mutagens not only alter DNA sequence but also influence the fixation of the altered sequence through modulation of gene expression, as well as understanding the influence of the status of apoptotic pathways on the ability to recover mutations.

FY99 GOALS

1. To develop and validate sensitive and predictive *in vitro* systems to identify, quantify and understand mode of action of potential human toxicants, especially carcinogens and mutagens.

2. To develop and validate sensitive and predictive *in vivo* systems to identify, quantify and understand mode of action of potential human toxicants, especially carcinogens and mutagens.

The approach to the first goal is to utilize relevant *in vitro* systems to evaluate risk to the human genome. In order to accomplish this, the division chose to characterize the human lymphoblastoid cell lines, AHH-1 and L3, and various subclones transfected with human cytochromes P450 cDNAs for evaluation of chemicals of interest to the FDA and the NCTR. These systems are highly relevant because they are expected to mimic human metabolic activation of specific mutagens and carcinogens and to provide sensitive endpoints for assessment of cytotoxicity and mutations at both autosomal and X-linked loci. Also, these systems lend themselves to molecular analysis making cross-species comparisons possible.

The approach to the second goal is to use nontransgenic and transgenic rats and mice to evaluate mutagens and carcinogens of interest to the FDA and NCTR. These *in vivo* systems are expected to complement and perhaps eventually replace the present *in vivo* rodent genotoxicity assays because their heightened sensitivity could obviate tests

at high doses where cell toxicity and mutagenicity become predominant. They provide a means to evaluate damage to critical genes in the target organ as well as in surrogate organs. And they provide one of the few means by which mechanistic questions can be addressed in an *in vivo* system. Also, they provide the ability to make critical evaluations and comparisons of molecular alterations in the transgene, endogenous surrogate genes and cancer genes allowing a more direct comparison to the molecular events described in human cancer.

Significance to the FDA

Human diseases are associated with spontaneous or induced somatic and germ cell mutations. Identification and quantitative measurement of the potency of suspected carcinogens and mutagens are essential to the FDA for regulating exposure of humans to harmful agents. The systems developed and characterized by the Genetic Toxicology Laboratory are capable of simulating the human condition, increasing the ability to detect weak carcinogens and decreasing the time required for determining a chemical's genotoxic potential. More importantly, the data generated will provide mechanistic information regarding the mode of action of the chemical being proposed for marketing and, therefore, a more accurate assessment of the potential risk to the human population.

CALORIC RESTRICTION GROUP

Introduction

This group has shown that 40% calorie restriction (CR) significantly alters the efficacy of many physiological processes in model rodent systems. A number of physiological, biochemical and morphological biomarkers that respond to this paradigm have been developed. It is now important to address two primary issues of interest to the NCTR and FDA: 1) developing practical methods for implementation of these findings relative to product testing and evaluation; and 2) determining the applicability of the group's previous findings to assessments and estimation of health risks in humans.

FY98 Accomplishments and FY99 Plans

Based on the recommendations of an advisory group at a national meeting to develop new guidelines for the use of the chronic bioassay in drug toxicity studies, a new research protocol (E6924.01) was drafted in collaboration with CFSAN to resolve several major problems that continue to plague the field of toxicological research. Some of these problems were the increased weight and obesity of test subjects, resulting in high mortality rates in rodent strains that are routinely used in the bioassay; the poor survival potential on purified diets, such as the AIN-76, that use casein as their protein source; and the use of dietary restriction (DR) to increase survival and reduce individual variation. The main purpose of this FDA-relevant protocol was to develop new methodologies and a comprehensive scientific database that will be used to support future NTP and FDA studies.

The physiological testing phase of Experiment 6924.01 was started in FY98 after a new data acquisition and process control system was developed to support these studies. Several major findings were discovered in the past year that have a major impact on FDA-related research and the chronic bioassay. The results of a two-year study to develop survival curves for the NCTR CD rat (a Sprague Dawley caesarian derived strain) clearly show that this strain has the best survival potential of any Sprague Dawley strain tested to date (35% mortality rate over two years), and that this strain is an excellent rodent model for use in future toxicological studies. Additionally, the mortality rate for the CD rat was reduced to 10%, 7% and 0% when the total caloric content of the diet was reduced by 10%, 25%, and 40%, respectively. These startling results are of major importance to the FDA and several large pharmaceutical companies that are planning to use DR for drug testing. These data suggest that even small changes in diet can dramatically increase longevity and decrease or delay the onset of age-related diseases, thereby changing the baseline sensitivity of the chronic bioassay. These survival data, together with body weight data for this species, will be used by the FDA to develop uniform regimens to control animal diets in future drug toxicity studies.

Significant changes in important physiological parameters, such as decreased body temperature and motor activity, as well as changes in intermediary metabolism (respiratory quotient), characterized by increased lipid metabolism during periods of fasting, were found at all three levels of dietary restriction (10%, 25%, and 40%). A group of rats from the various nutritional groups were sacrificed and were sent for histopathology. A complete set of blood chemistries was done on these animals in the past year. Many parameters such as triglycerides, blood glucose, free fatty acids, etc., were found to be altered by DR in all of the test groups. Since previous studies have shown that changes in these variables are accurate and sensitive biomarkers that can be used to predict drug toxicity, the preliminary results of this study suggest that the pharmacodynamics and pharmacokinetics of drugs will be significantly altered by all three levels of DR. Additional evidence that diet modulates drug efficacy was derived from animals given the drugs ketamine and xylazine to anesthetize animals prior to implanting temperature transmitters. Surprisingly, we found that the time to onset of sleep was decreased by DR and that the depth and duration of anesthesia was significantly increased from 2.5 hours to 7.5 hours in DR rats, clearly indicating that metabolism of the drugs was altered by diet.

Another purpose of this experiment was to compare the survival potential of a new purified diet (AIN-93M), a formula that was recently developed by CFSAN, with the natural formula NIH-31 diet. Although the study has not been completed, the preliminary results (75 weeks of age) suggest that the survival rate of rats raised on the AIN-93M diet is very similar to the NIH-31 formula, a diet that has been used in previous studies to promote extended longevity. These results may be extremely important to the FDA because it shows for the first time that purified diets such as the AIN-93M have excellent survival characteristics and that they can be used successfully in the chronic bioassay.

Finally, a new noninvasive method was developed in the past year to accurately determine body composition in laboratory rodents using magnetic fields (EM-SCAN device). This procedure will help to predict the potential risk of exposure to FDA-regulated drugs that are preferentially stored or metabolized in specific body tissues such as fat and muscle (lean mass). Additionally, the results from this study will allow the development of accurate dose response curves for test groups with different body compositions.

The data and methodologies that have been developed in the past year provide practical solutions to several complex problems that confront the FDA related to the use of the chronic bioassay for drug testing. Also, they will be used to determine basic mechanisms by which drugs alter the aging process and the onset and proliferation of age-related diseases.

The laboratory is attempting to determine if physiologic, metabolic, biochemical and molecular changes that occur with caloric restriction in rodents change in similar ways in humans who have undergone a surgical procedure that produces caloric restriction (CR). This is a three-year study with a two-year follow-up. The goal is to obtain blood

prior to gastric bypass surgery, biopsy tissues and blood at the time of surgery, blood at post-operative intervals, and biopsy tissues approximately one year after the initial bypass surgery (during a "tummy tuck" surgery) from 30 to 50 morbidly obese female patients.

The study has recently passed the one-year mark and 17 patients are now on study. At this rate, it is anticipated that the minimum target number of 30 patients will be reached around the end of the second year. Thus, the ideal number of 50 patients on study should be easily approached prior to the end of the third year. All follow up could then be completed in only one year after the final patient is entered into the study. During the first year of the study all procedures for optimal blood and tissue preparation and distribution were put in place. These procedures include "batch" red blood cell and lymphocyte isolation, followed by preparation for either immediate analysis, culturing or storage according to the individualized specifications of each investigator. Similar procedures have been successfully put into place for biopsied tissues where individualized specifications must be met. The National Institute on Aging (NIA, Health ABC Program) is considering funding a large number of human studies that would require this type of cell and tissue handling. The successful implementation of these procedures has led to an NIA request for the labs consultation in set up of procedures for such future studies.

Detailed procedures have been optimized for physiological evaluation of each patient, and a comprehensive computer networking system has been put in place which allows for interaction among clinical, physiological, metabolic, biochemical and molecular data bases. Since physiological data are the first data collected on a patient, encouraging preliminary results have been obtained. For example, it is now clear that body temperature is lower in the calorie restricted state, and the circadian pattern indicates that the highest temperatures are during the active period while lowest values occur during sleep. Additionally, the amplitude of change is significantly higher in the thin women. These findings (along with O_2 and CO_2 levels, and resultant RQ values) are similar to observations made in ad libitum (AL) and CR rodents.

Since post-operative tissues have only recently begun to become available for some of the earliest patients placed in the study, data evaluation is just beginning. However, several interesting and encouraging preliminary observations have been made. For example, it appears that accumulation of DNA damage as measured by the comet assay are higher in the obese state, and in a preliminary study, induction of heat shock proteins was higher in red blood cells from thin women. These findings match results seen in AL and CR rodents. Early observations concerning rates of apoptosis, spontaneous mutation frequency, and fat cell culture also seem to mimic results seen in AL and CR rodents. Detailed analysis of oxidative phosphorylation in mitochondria from lymphocytes has been completed. Activity of complex III of electron transport in lymphocytes from obese women was found to be over twice that in thin women. This surprising finding can be better understood in light of appropriate kinetic evaluation, and Vmax was calculated to be significantly higher in obese women. Since Vmax is a derivative of total enzyme, this observation suggests that lymphocytes from obese

women contain larger quantities of complex Ill. However, Km (a binding constant which indicates binding affinity of substrate to the active site of the complex and is a measure of enzyme efficiency) for obese women was 1.7mM ubiquiol-2, while the Km was 0.69mM ubiquinol-2 for thin women (p>O.000 1). This highly significant difference indicates that electron transport can operate at a much more efficient level in lymphocytes from thin women. Additionally, the higher levels of total protein indicated by Vmax in obese women appears to be an attempted compensation for a compromised enzyme system. The higher Km in obese individuals produces a barrier to electron flow and this suggests a potential mechanism for increased free radical production associated with obesity, development of degenerative diseases and aging. These results are similar to findings in AL and CR rodents.

Work concerning numerous other measures will become available in the near future as additional tummy tuck samples are collected.

FY99 Goals

1. Determine the ideal level of caloric intake and develop methods for implementation of animal studies.

2. Evaluate a human model system virtually identical to the animal experimental system to elucidate the impact of dietary intake on a number of physiological, biochemical, metabolic and molecular endpoints.

Significance to the FDA

Experiment E06924.01 is a collaborative study between the NCTR and CFSAN that is designed to resolve problems associated with the chronic bioassay and risk assessment. Preliminary data indicate that a 10% reduction in calories increases survival rates, reduces individual variability, and spontaneous tumor incidence. The FDA will be able to use data such as these to more accurately establish guidelines for performance of bioassays. Experiment E06998.01 provides a unique opportunity to evaluate numerous biomarkers that will provide important baseline data to test the efficacy of calorie restriction humans, and will establish relationships between nutritional status and human health. Further, this study will establish standards concerning the sensitivity of obese versus non-obese populations to drugs. Since many decisions concerning human health are based on rodent data, our studies will provide important baseline data, derived from a variety of human tissues, useful for improving the reliability of extrapolation of rodent data to humans in any number of situations important to the FDA.

REPRODUCTIVE TOXICOLOGY LABORATORY

Introduction

Research support scientist, Stacey Dial, is assaying estrogen receptor and protein binding properties of putative endocrine disruptors

Increasing recognition of the importance of women's health issues re-emphasizes the need for better identification of developmental toxicants and improved assessment of their risk. Congenital malformations recognized at birth affect one in 14 infants (7%); this doubles when later recognized deficits are included. Some experts estimate that at least one child in three has a birth defect. Additionally, another 7% of infants have low birth weights and at least 25% of recognized pregnancies end in spontaneous abortion.

Birth defects cause over 20% of all infant deaths and are the fifth leading cause of potential years of life lost. More money is spent by states on developmental disabilities (including mental retardation) than on any other category of chronic disease. Over one dozen chemicals, the majority of which are FDA-regulated, are recognized as human teratogens; many more agents are suspected human teratogens. However, no chemical regulated by FDA has been tested for developmental toxicity in pregnant women; only recently have non-pregnant women been included in clinical trials, and some consideration is now being given to also including pregnant women. This puts a heavy burden on laboratory animal research.

FY98 Accomplishments and FY99 Plans

Over the past 15 to 20 years, NCTR has been a leader in defining the normal and estrogen-altered reproductive tract developmental profile in the rat. This expertise provided the foundation for the reproductive and developmental toxicology involvement with the FDA Office of Women's Health initiatives. This same expertise and the well-defined estrogenic database created over the past 20 years has led to the initiation of a project to create and validate a computerized knowledge base utilizing experimental data to aid in the regulatory decision process, funded by a series of grants from FDA's Office of Women's Health. Nine (9) papers on Quantitative Structure Activity Relationships (QSAR) models for estrogen and related activities have been published this fiscal year. QSAR models are being developed for estrogen binding to rodent alpha fetal protein (AFP) and human testosterone estradiol binding globulin (TEBG). Sixty-five (65) chemicals have been evaluated in an estrogen receptor assay. This latter activity is being partially funded by a CRADA with the Chemical Manufacturers'

Association (CMA). The derived data will be used to assess the QSAR models being developed in collaboration with ROW Sciences, Inc. Additionally, scientists within the laboratory are collaborators with Dr. Fred vom Saal (University of Missouri - Columbia) in a research project on endocrine disruptors.

Estrogen Knowledge Base contributors have participated in several national and international meetings as invited speakers and have served as members of the Endocrine Disruptor Screening and Testing Committees' (EDSTAC) screening and testing workgroup.

Major studies involving several NCTR research divisions on several endocrine disruptors are underway. Estrogenic chemicals in foods, devices, drugs, veterinary medicines, and other FDA-regulated products are a developing concern. NCTR has taken a leadership role in the area, both within and outside FDA.

Classical Segment II teratology studies of fumonisin B_1 (FB_1) in rats and rabbits were completed. Abnormalities were observed only at concentrations that produced maternal toxicity. The ratio of sphinganine to sphingosine, which was used as a biochemical marker of FB_1 exposure, was increased in several maternal tissues but was not altered in fetal tissues. This suggests that FB_1 may not have crossed the placenta and further suggests that, in the absence of maternal toxicity, this compound does not appear to be a significant developmental toxicant. Three manuscripts detailing this work were published in FY98.

Folic acid has been demonstrated to decrease the recurrence and occurrence of neural tube defects in humans. Several studies have shown that this is not due to a reversal of a dietary deficiency of the vitamin and have further suggested that folate metabolism may be altered in some individuals leading to a failure of neural tube closure in the offspring. In order to investigate the role of 5,10-methylenetetrahydrofolate reductase (MTHFR) in producing neural tube defects, the lab used antisense oligonucleotides to knock out activity of this enzyme in mouse embryos. It was found that neural tube defects were produced in embryos with decreased activity of MTHFR. When 5-methyltetrahydrofolate was co-injected with the MTHFR antisense oligonucleotide, the incidence of neural tube defects was decreased compared to the group injected with the antisense only. When methionine was co-injected with the antisense, there was no change in the number of embryos with neural tube defects. These findings suggest that MTHFR may play a role in normal closure of the neural tube and that decreases in activity of this enzyme may be involved in neural tube defects. These findings further suggest that supplementation with exogenous folic acid is able to overcome such decreased enzyme activity. This work is currently being written for submission for publication.

The lab has also investigated the role of the folate receptor in neural tube closure. When mouse embryos were injected with an antisense oligonucleotide for the folate receptor, neural tube defects were produced. The lab is currently continuing to collect data on this project and hope to complete it during this year.

The anticonvulsant drug valproic acid (VPA) is known to produce neural tube defects in 1% to 2% of exposed human offspring as well as in animal models. The mechanism for this effect is unknown, but it has been postulated that VPA might alter expression of cell adhesion molecules in the neural tube during fusion. Western blotting techniques demonstrated that there was no change in the overall expression of three isoforms of N-CAM (Neural-Cell Adhesion Molecule); however, an isoform with a slightly higher molecular weight was induced by VPA treatment. The role of this isoform in neural tube closure is unknown. Recently, immunohistochemistry and *in situ* hybridization were used to determine that localized alterations in expression in cranial neuroepithelium do occur following treatment with VPA, suggesting that VPA-induced developmental toxicity may involve localized defects in cellular adhesion. This work is currently being readied for submission for publication.

A molecular biology capability has now been brought to this research area which enables developmental toxicity to be measured in terms of effects on embryonic, fetal and neonatal gene expression. One approach which the laboratory has developed involves testing a group of candidate genes (selected because they are regulated by the drug of interest and are important in the mechanism of the drug's effects on *adults*) to determine which are expressed during development. Genes that match these criteria are then used as "developmental biomarkers" for biologically significant prenatal drug exposure. Drug treatment of pregnant rat dams is followed by analysis of fetal tissues using sensitive molecular assays to determine whether fetal expression of developmental biomarker genes was affected. Previously, the laboratory successfully used this approach to identify a specific insulin-regulated gene (insulin-like growth factor [IGF] binding protein-1) as responding in fetal liver to maternal insulin levels and insulin-dependent diabetic status, and as being part of the mechanism by which fetal growth is retarded in mothers with insulin-dependent diabetes. In FY98, they extended this approach to study noninsulin-dependent diabetes and a drug recently approved for its treatment (troglitazone). Troglitazone causes fetal growth retardation in rodents and is not recommended for pregnant women. Beginning with a group of fifteen candidate genes that are regulated by troglitazone in adults and whose products are important in lipid or carbohydrate metabolism, the lab demonstrated that nine of these genes were expressed in fetal adipose tissue, liver and/or pancreas and, therefore, are potential developmental biomarkers for troglitazone. During FY99, the lab plans to determine the effects of maternal noninsulin-dependent diabetes and troglitazone treatment on fetal growth and fetal expression of these nine developmental biomarker genes, and thereby identify a mechanism by which troglitazone induces fetal growth retardation.

Estrogens are developmental toxicants, and some effects of estrogens are mediated through IGFs and IGF binding proteins. Based on previous demonstration of tissue-specific expression of estrogen receptor-α, IGF-I and IGF binding protein-1, -3, -4, -5 and -6 within neonatal rat uteri, in FY98 the lab tested whether exogenous estrogens (diethylstilbestrol and tamoxifen) and antiestrogens (ICI 182,780) would alter mRNA expression of these genes. The lab found that exogenous estrogens induced specific immediate and long-term alterations in expression and regulation of IGF-I, IGFBP-3 and

IGFBP-4 consistent with their involvement in the mechanism of exogenous estrogen developmental toxicity, and these effects on gene expression were blocked by antiestrogen treatment. During FY99, the lab plans to extend these studies to analyze exogenous estrogen effects on neonatal rat ovaries.

Based on gene knockout experiments, teratogenic effects of retinoic acid on limb skeletal growth and morphogenesis were predicted to be mediated through a specific receptor molecule, Retinoid X Receptor-α (RXRα). Previously, this laboratory demonstrated for the first time that RXRα mRNA was expressed in fetal limb skeletal precursors. In FY98, the lab extended these findings by demonstrating strong skeletal expression of RXRα mRNA in limbs of retinoic acid-exposed fetuses, consistent with the knockout results. They also established culture conditions in which isolated embryonic limbs develop *in vitro*. During FY99, they plan to assay development in cultured embryonic limbs treated *in vitro* with teratogens and IGFs, as well as using specific antisense oligonucleotides to block limb expression of specific genes. By this approach, they plan to elucidate molecular mechanisms of teratogens that interfere with limb development.

FY99 Goals

1. Develop improved methods and new strategies for detection and prediction of developmental toxicity in laboratory animals and the human population, focusing on reproductive tract development, whole embryo development, pharmacokinetics during development, and the molecular biology aspects of development.

2. Develop a knowledge base for the estrogenic action of xenobiotics during development.

The availability of natural and synthetic estrogens, as well as anti-estrogens (each with different pharmacological and toxicological properties), provides opportunities for development of methods and mechanistic approaches to predict risk. Estrogens are etiological agents in female reproductive tract toxicity, a major human health problem. Exposure to FDA-regulated estrogens and anti-estrogens occurs in tens of millions of women. There is oral contraceptive exposure in over 100,000 pregnancies each year. In the U.S., about 5% of women will receive tamoxifen sometime during their lifetime. The fertility drug, clomiphene, is responsible for 1% of the live births. Phytoestrogen exposure of the human population via food is virtually universal; infants consuming soy formula are exposed to the highest doses. Some environmental chemicals, such as plastics and pesticides, possess estrogenic activity and are found in FDA-regulated products. Estrogens are studied both with respect to their varying pharmacological and toxicological properties and their common mechanism of action. The laboratory is constructing an estrogen knowledge base to predict hormonal activity of untested xenobiotics and to help generate hypotheses identifying gaps in regulatory data. These strategies are important in providing FDA with human and computational expertise and experimental flexibility in dealing with regulatory issues in these areas.

Women and their embryos/fetuses are exposed to a number of xenobiotics during pregnancy; most drugs are necessary to maintain maternal health and well being. Mechanistic studies provide strategies and new concepts to help identify at-risk pregnancies as well as suggest possible intervention therapies (*e.g.*, the FDA issue of folate supplementation) that could circumvent developmental toxicity. Species and strain differences can be investigated *in vivo* (*e.g.*, Segment II developmental toxicity studies), in which maternal physiological factors can be monitored to determine any maternal effects of a chemical. Maternal plasma and embryonic/fetal drug levels can be measured to estimate embryonic exposure. Toxicity assessments in an *in vitro* whole embryo culture system allow for the evaluation of the effects of a chemical (or metabolite) in the absence of possible confounding maternal effects.

As part of NCTR's strategic move into the molecular biology of development, efforts toward identifying potential gene biomarkers critical to development are underway. One such effort involves insulin-like growth factors (IGFs), IGF binding proteins, and genes regulated by the antidiabetic drug troglitazone, all of which appear to be involved in the mechanism of maternal diabetes. Since maternal diabetes increases the incidence of both abnormal fetal growth (associated with increased health risks to mother and child) and birth defects, these molecular probes may be important in the etiology of such birth defects and abnormal fetal growth.

Significance to the FDA

Reproductive and Developmental Toxicology is investigating the effects of drugs and other xenobiotics regulated by FDA which collectively have extensive human exposure during pregnancy. By improving methods to detect and characterize developmental toxicants as well as determining the mechanisms for their effects, the FDA will be in a better position to predict the human developmental toxicity of regulated products and to advise regulated industry of appropriate procedures. This research area utilizes an integrated research approach by emphasizing molecular, endocrine, limb and whole embryo *in vitro*, and pharmacokinetic techniques.

This combination of techniques and expertise is unequaled by any other single group in the FDA for studies in developmental toxicity and positions the individual scientist to be able to best contribute to the FDA regulatory arena.

MICROBIOLOGY

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Dr. Doug Wagner operating a Perkin Elmer ABI PRISM 310 Genetic Analyzer

Introduction

Microbiology is an exceptionally broad discipline encompassing research areas as diverse as taxonomy, physiology, bio-chemistry, molecular biology, pathogenesis, food and industrial microbiology, and ecology. In fact, modern biotechnology rests upon a microbiological foundation. The microbiology research at the NCTR serves a multipurpose function with specialized expertise to perform fundamental and applied microbiology research in areas of the FDA responsibility. The microbiology research also responds to microbial surveillance and diagnostic needs for research projects within the Agency. The major aims of the microbiology research are to raise the general awareness of the importance of microorganisms in public health and to provide data to improve our understanding of the mechanisms by which toxic events occur in humans. The research is organized to handle many aspects of microbial toxicology and continue to train staff to meet the research and regulatory needs of the FDA. The microbiology research at NCTR is divided into five focal areas with strategies and objectives unique to the problem posed. Goals and accomplishments for each focal area are discussed separately below.

FY98 Accomplishments and FY99 Plans

In FY98, microbiology-related research issues were discussed with microbiologists from other FDA centers and field laboratories. The scientific exchange led to the initiation of new projects. A short summary of some resulting collaborative research projects is listed below:

1. Development of quantitative assays for measuring the tuberculocidal activity of chemical disinfectants.

Tuberculosis, once considered a disease brought under control through use of antibiotics, has re-emerged as a serious health concern in the United States. The percentage of tuberculosis cases caused by strains of *Mycobacterium tuberculosis* that are resistant to one or more of the antibiotics used in therapy is increasing. While tuberculosis is not readily transmissible by casual contact, it can be spread where individuals live or work in very crowded conditions, and perhaps by certain medical procedures as well.

Numerous chemical agents are used to disinfect and sterilize medical instruments, such as endoscopes, that cannot be autoclaved. Endoscopes contain crevices and channels that are difficult to clean and can harbor bacteria. Many of the liquid chemical germicides on the market claim the ability to kill *Mycobacterium tuberculosis*, yet improperly washed and disinfected endoscopes have been linked to the transfer of this organism from tuberculosis patients to previously uninfected individuals. This has raised the concern that some of the disinfectants may not be fully effective under the prescribed conditions.

The FDA is preparing to evaluate the tuberculocidal activity of a large number of liquid chemical germicides. The NCTR Division of Microbiology has been instrumental in the preparation for this evaluation by developing the expertise required to perform the Association of Official Analytical Chemists (AOAC) tuberculocidal assay, clarifying and expanding the protocol for this assay, and training Office of Regulatory Affairs (ORA) personnel to conduct this assay at their own facilities.

The current methods for determining the tuberculocidal activity of disinfectants are difficult to perform, poorly reproducible, and require up to 90 days to obtain results. Scientists in the laboratory are implementing molecular methods (E06965.01) to both improve the sensitivity and accuracy of the test, and shorten the time required for a definitive answer. Using a mycobacterial strain carrying the firefly luciferase gene, they have developed a rapid, quantitative method for determining tuberculocidal activity by detecting light produced by bacteria that survive exposure to disinfectants. Because this method does not require the mycobacteria to grow significantly after the disinfectant exposure, the assay time is reduced to one day--compared with weeks to months for the other tuberculocidal methods. This method has been tested against several different disinfectant chemistries, and shows promise in greatly reducing the time required to test the large number of liquid chemical germicides on the market. NCTR scientists will continue to improve this method, screening different test organisms with better growth characteristics and testing a broad range of disinfectants.

2. Development of methods for the detection of foodborne pathogens.

Despite the fact that the United States food supply is the safest in the world, tens of millions of cases of foodborne illnesses occur in the United States every year with a cost to the economy of an estimated 1 to 10 billion dollars. Therefore, the microbiological safety of food has become an important concern of consumers, industry and regulatory agencies. The U.S. Food and Drug Administration gives a high priority to protecting the public from microbial contamination of the food supply. The research program in the Division of Microbiology in FY98 had a project (E06988) to develop molecular methods to detect and identify foodborne bacterial pathogens. In addition, scientists in the Microbiology Laboratory collaborated with scientists in the Division of Chemistry to use mass spectrometry methods for the rapid identification of bacteria (E06785 and E06931).

A protocol (E06988.01) for the detection of 13 species of foodborne pathogens in foods using the polymerase chain reaction (PCR) technique was developed in FY98. The method used a universal enrichment medium and the same PCR conditions with 13 sets of specific primers for the detection of foodborne pathogens. The foodborne pathogens examined were *Escherichia coli*, *Shigella*, *Salmonella*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, *Vibrio cholerae, V. parahaemolyticus*, *V. vulnificus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus*. No interferences were observed using the PCR assay for food samples artificially inoculated with each single bacterial species.

In collaboration with the Division of Chemistry (E07005.05), rapid methods of identifying whole bacteria by their mass spectra are being developed. Rapid identification is needed for FDA inspectors to make quick decisions on whether to allow the sale of food products that may harbor pathogenic bacteria. Nitrosoguanidine and UV mutagenesis were used to produce mutant strains of bacteria differing from the wild types in a single gene for toxin resistance so that the abilities of mass spectral methods to differentiate closely related bacterial strains could be compared. MALDI-TOF mass spectrometry, in conjunction with amino acid sequencing, was used to identify two of the principal ions that were detected by mass spectrometry in cultures of *Escherichia coli* and *Shigella flexneri* as derived from the highly abundant acid-resistance proteins HdeA and HdeB.

In another collaboration with scientists in the Division of Chemistry (E06931.01) and the Colorado School of Mines, pyrolysis-mass spectrometry and MALDI-TOF mass spectrometry are being used for rapid bacterial identification by analyzing the bacterial chemical composition and for evaluating the roles of physical, chemical and environmental conditions on the bacterial recognition patterns. The cell components that generate patterns observed in MALDI/TOF mass spectrometry were identified to be low- molecular-weight-proteins, that could be used for amino-acid-sequence-analysis and confirmation of bacterial identification using data stored in the Gene Bank.

The survival of *Shigella* species on prepared foods that require no heating before consumption was evaluated on various salads. Four different salads supported the survival of bacteria under cold storage, one of them as long as 20 days. A method was developed for the rapid detection of *Shigella spp*. in salads. Since salads contain soluble and particulate ingredients, an elution-and-filtration method was devised to separate bacteria from salad materials and PCR was used for the bacterial identification. Using centrifugation, filtration and enrichment, *Shigella flexneri* was identified from four different vegetables and salads following PCR amplification.

Aeromonas spp. are important organisms listed in the FDA pathogen list. *Aeromonas spp*. are commonly found in a wide range of aquatic systems and foods and have been isolated from coastal waters, lakes, rivers, drinking water, and a variety of foods. These organisms cause traveler's diarrhea, acute diarrhea or dysentery in children, adults and older people that can be severe and even life threatening. Because *Aeromonas* can resemble *E. coli* on several media, the incidence and the importance of this microorganism have been underestimated. It is a major concern for FDA because the

U.S. imports food from developing countries where incidences of *Aeromonas*-associated gastroenteritis are much higher and because it is indigenous to temperate estuarine areas. The identification of *Aeromonas* isolates to the species level by using only phenotypic methods is impractical because of confused taxonomy. A 16S rDNA-based PCR method was completed in FY98 for the detection of *A. caviae* and *A. trota* from seafood and water samples in less than eight hours.

Little was known about the aerolysin toxins and their regulation. The molecular cloning of the aerolysin (toxin genes) and nucleotide sequences of these genes will allow us to develop a specific probe for toxin-producing strains and study the structure and regulation of these genes in pathogenic organisms. In order to determine the mode of action of bacterial toxins on cell lines, to study antigenic properties, and to develop immunodiagnostic kits against these toxins, it is important to have highly purified toxins. The Division developed in FY98 a rapid method for producing pure toxins by cloning various toxin genes onto a His-tag expression vector, which is commercially available. This His-tag purification procedure has several advantages over conventional purification methods; for example, large amount of toxins can be purified in much less time using very few steps and chemicals.

A collaborative project (E07001.01) has been initiated with the FDA Gulf Coast Seafood Laboratory (GCSL) staff, Dauphin Island, AL. The GCSL has found that the supernatant fraction of centrifuged oyster homogenate is lethal to *V. cholerae* and *V. vulnificus* and can interfere with detection of these pathogens in oyster samples. Identification of lethal substances may benefit detection methods and/or lead to antibacterial treatments of seafoods. While cholera occurs primarily in developing countries, it is a major concern for FDA because the U.S. imports food from countries with endemic cholera and because it is indigenous to temperate estuarine areas including the Gulf of Mexico. Toxigenic *V. cholerae* was found on a number of occasions in oysters from Mobile Bay in 1991 and 1992 by both FDA and the Center for Disease Control and Prevention (CDC). The antibacterial activity of oyster supernatant was also found to be exhibited against *V. vulnificus* but not other *Vibrio spp*. tested. The density-dependent collapse of the *V. cholerae* population may be consistent with phage infection or agglutination; either of which would be a novel finding for the bacteriological detection procedure for food products. The identity and mode of action for these antimicrobial agents need additional study since these may have considerable ecological and public health implications for the occurrence and control of pathogenic *Vibrios* in oysters. Inactivation of naturally occurring antimicrobial agents in shellfish also may be an effective strategy for improving detection methods for pathogens. Another potential benefit of this research would be to utilize these antimicrobial agents in food processing to reduce the risk of foodborne bacterial infections.

Since oysters serve as the reservoir for several *Vibrio spp*., it was thought that they produce an antibacterial protein that is lethal to *V. cholerae* and *V. vulnificus*. But no such protein has been isolated to date. It could very well be that the phages specific to *V. vulnificus* and *V. cholerae* killed these bacterial strains. Indeed, several phages were later isolated from oysters. These phages, especially the ones with a broad host range

could be used to develop a phage-luciferin-luciferase or phage-green-fluorescent-protein based reporter system to detect the pathogenic *V. vulnificus* in seafoods. However, since there is not enough biochemical and genetic information available about these phages, their genetic and biochemical characterization is necessary to develop such a reporter system. In FY98, the Division has been successful in large-scale purification of a broad host range *Vibrio vulnificus* phage that grows on strains A-9 (moderately virulent environmental isolate), J-7 (a virulent environmental isolate, VBNO [isolated from a blue crab, *Callinectes sapidus*] and MO6-24 (human primary septicemia blood isolate). Several other phages that did not have a broad host range were also ultrapurified. In order to initially characterize these phages, their morphological characterization was done by electron microscopy, and their structural components were analyzed by sodium dodeca sulphate polyacrylamide gel electrophoreses (SDS-PAGE). Their developmental intermediates were also isolated by ultracentrifugation and studied by electron microscopy. In order to carry out their genetic characterization, their genomic DNA was isolated and subjected to a battery of restriction endonucleases. The physical and genetic mapping of at least one of the broad host range phages is a necessary first step towards the achievement of a long-term objective to develop the phage- and fluorescence based-reporter system. The initial physical mapping studies done here will be helpful in achieving this goal.

During FY98 an interagency Food Safety Initiative was developed. Several projects were initiated with scientists from CVM. Some of our initial results are described below:

Studies on the mechanism of fluoroquinolone-resistant *Salmonella spp*. isolated from animal (poultry) feeds and the animal production environment and the development of molecular methods for screening the drug-resistance genes are being conducted (E07048.01).

Salmonella spp. is the leading source of food-borne illness throughout much of the world. For example, between 1973 and 1987, 51% of human food-borne bacterial disease cases in the United States were caused by *Salmonella spp*. It has been estimated that the human illness due to food-borne pathogens costs U.S. consumers between 4.5 and 7.5 billion dollars each year in medical costs and lost productivity. Poultry products are consistently identified as important sources of *salmonellas* that cause illness in humans. Several studies have correlated the use of antimicrobial agents in veterinary medicine with the emergence and spread of fluoroquinolone resistance among *Salmonella* strains, with potentially serious effects on food safety and on the treatment of both human and veterinary health disorders. Two species of *Salmonella*; *S. typhimurium* and *S. enteritidis*, are frequently isolated from poultry and cause disease to humans. Several studies have reported the isolation and characterization of fluoroquinolone resistant *Salmonella spp.* from poultry and most of these studies used the Minimum Inhibitory Concentration (MIC) procedure for antibiotic resistance. However, antibiotic resistance data from these studies are unreliable because MIC methods often give ambiguous results. These results must be confirmed by genetic analysis to show the presence of mutations on genes responsible for drug resistance. The amplification of the gyrA gene by PCR and its restriction analysis will

give definitive results and can be used to screen large samples. Several factors influence the determination of the percentage of *Salmonella* resistant to the break-point concentration of fluoroquinolone (>1 μg/ml). The veterinary isolates of *Salmonella* highly resistant to nalidixic acid (>512 μg/ml) have been also found to be resistant to fluoroquinolones and the MICs were similar to those for clinical isolates from patients who failed to respond to ciprofloxacin therapy. The increasing incidence of quinolone-resistant isolates of *Salmonella* is clearly a potential health risk, possibly compromising effective antibiotic therapy with fluoroquinolones. These findings have drawn the attention of regulatory agencies, such as FDA and USDA. The drug resistance breakpoint differs from country to country, as do the expertise of the laboratories to isolate fluoroquinolone resistant *Salmonella spp.* and the frequency of veterinary drug use on the farm. The data on the percentage of fluoroquinolone-resistant *Salmonella spp.* is not available in the U.S. The baseline data obtained from this study will be helpful for monitoring the fluoroquinolone resistance in *Salmonella spp*. due to variation in temperature and application of veterinary drugs. This study will allow the Division to find the difference between the epidemic outbreak of drug (fluoroquinolone) resistance in *Salmonella spp.* as compared to normal occurrence and the effect of application of fluoroquinolones in poultry. In addition, elucidation of the molecular mechanism(s) for quinolone resistance in *Salmonella spp*. from this study will also allow the development of rapid methods for routine surveillance. The findings can then be used to make necessary decisions by the FDA in regulating the use of fluoroquinolones in the poultry industry.

Erythromycin is extensively used for the control of *staphylococcosis*. The prevalence of the erythromycin resistance genes (erm) in *Staphylococcus sp.* isolated from diseased chickens was determined (E06901.01). Forty-six (46) erythromycin-resistant *Staphylococcus spp.* were isolated from diseased chickens. A majority of the isolates were resistant to high concentrations of erythromycin, oleandomycin, spiramycin and tylosin. Thirty-four (34) of these isolates were coagulase-positive *S. aureus* and the remaining twelve (12) were coagulase-negative. Dot blot hybridization indicated that ten of the twelve coagulase negative strains harbored the ermA gene. Similar analysis also indicated that 22 of the 34 coagulase positive *Staphylococcus spp.* harbored the ermA gene. The ermA gene was exclusively found on the chromosome. Two different ermA EcoR1 restriction-fragment-length polymorphisms were identified. A majority of the ermA- positive strains had two ermA inserts, viz. A 8.0 and 6.2 kb EcoR1 fragments. A few strains had a 6.3 and 5.8 kb EcoR1 fragments. Plasmids (2.0-16.0 kb) were present in all the isolates. Southern hybridization indicated that only two of the 12 coagulase-negative *Staphylococcus spp.* contained the ermC gene on the plasmid. Twelve (12) of the 34 strains of *S. aureus* contained the ermC gene. Eleven (11) of these strains had the ermC gene on a 2.5 Kb plasmid and one strain had the gene on a 4.0 kb plasmid. Results indicate that either ermA or ermC was present in all isolates and that ermA was the dominant gene in coagulase-negative and coagulase-positive avian *Staphylococcus spp.*

In FY98, the Division of Microbiology established collaborative research agreements with CVM to assess the constituents of competitive exclusion products for activity and

antimicrobial drug resistance. As a result, a cooperative project (E07049.01) was established for FY99 and 2000 to develop methods for identification and *in vitro* efficacy determination of individual bacterial components in competitive exclusion products.

Another aspect of this competitive exclusion project is to determine if the bacteria have the potential to transfer antibiotic resistance to the human food supply. The resistance to vancomycin was unknown until 1988 and from that point onwards, the frequency of vancomycin-resistant bacteria is increasing every year. The use of vancomycin in humans and avoparcine in the cattle industry has resulted in several vancomycin-resistant bacteria. Since vancomycin is regarded as an antibiotic of last resort in treating infection with Gram-positive bacteria, and vancomycin-resistant bacteria are often resistant to multiple antibiotics, it is very important to monitor the drug resistance and its transfer to other bacteria. The use of antibiotics in humans to treat the infections can be limited but not completely avoided. By using the competitive-exclusion bacteria, the use of antibiotics in the poultry and cattle industry can be minimized. These bacteria colonize the intestine and prevent the colonization of other harmful bacteria that become the cause of human infections later. One such competitive-exclusion product is used in the poultry industry and contains a mixture of 29 bacteria. Some of these bacteria are highly resistant to vancomycin and may or may not be involved in the drug-resistance transfer.

In order to determine the role of competitive exclusion culture, the individual bacteria will be isolated and the mechanism of vancomycin resistance, which is not understood properly in these bacteria, will be studied. The method of bacterial conjugation, as mentioned above, will be used to see if these bacteria are involved in the transfer of drug resistance.

3. Assessment of the effects of food additives and drugs in food on the human intestinal microflora. Determination of the role of intestinal microflora in the metabolism of therapeutic drugs, food additives and cosmetics.

In recent years, questions have been raised concerning the consumption of low levels of food additives and antimicrobial residues in foods and the effect of these residues on the indigenous human intestinal microflora. Intestinal microflora are an essential component of human physiology because they act as a barrier against colonization of the gastrointestinal tract by pathogenic bacteria. They also play important roles in the digestion of food and the metabolism of drugs, xenobiotics and nutrients. Repeated exposure to antimicrobial residues and food additives may perturb the normal population density of intestinal microflora, altering enzyme activity for the metabolism of endogenous and exogenous substances, and impairing colonization resistance, which may increase susceptibility to infection by enteric pathogens such as *Salmonella*, *Shigella* and *Escherichia coli*.

The Director of the Division has provided guidance to scientists at the Center for Veterinary Medicine (CVM) and reviewed research protocols for the CVM on the effects of low levels of antimicrobial residues in food on the human intestinal microflora. In

addition, he wrote a guidance document for the World Health Organization on "Assessing the effects of antimicrobial residues in food on the human intestinal microflora." This document will be used by regulatory agencies, industry drug sponsors and the international scientific community as a guideline for making an assessment of the potential risk of dietary intake of residues of antimicrobial animal drugs.

Studies have continued in the laboratory on the determination of the role of intestinal microflora in xenobiotic metabolism (E06032.01). Various enzymes from the human intestinal tract play a role in the activation and/or detoxification of food additives, therapeutics, azo compounds, and nitro compounds. Some azo and triphenylmethane dyes are reduced to mutagenic compounds following reduction by bacteria from the human intestinal tract. Scientists in the laboratory are investigating the effects of bacteria from the human intestinal tract on seven different azo and triphenylmethane dyes currently used in the food, pharmaceutical, cosmetics, and aquaculture industries. All of these dyes were reduced by the bacteria isolated from the human intestinal tract. Mutagenicity assays, using two strains of *Salmonella typhimurium*, showed that none of the azo dyes or their reduction products were mutagenic. The azoreductase genes from the various anaerobic bacteria involved in the reduction of these dyes were analyzed, and variations were found among the structures of the azoreductase genes from the different bacteria.

Azoreductase and nitroreductase convert some therapeutic azo and nitro compounds to their activated forms. These drugs are used not only for the treatment of bacterial infections but also for the treatment of inflammatory bowel diseases with unknown etiology. The reductase activities in fecal samples from pouchitis patients during the onset of the disease and following recovery were evaluated. Higher levels of azoreductase and nitro-reductase were found in all of the patients following recovery. In addition, the role of anaerobic bacteria from the human intestinal microbial flora in the metabolism of nitro-substituted benzodiazepines, which are used extensively for the treatment of anxiety, was studied. These compounds have been shown to be teratogenic in experimental animals, and nitroreduction by anaerobic intestinal bacteria is considered to be involved in the mechanism of toxicity. The bacteria isolated from the human intestinal tract that had nitroreductase activity were shown to reduce the nitrazepam to 7-aminonitrazepam.

Ingestion of antimicrobial agents often induces resistance in microorganisms of the intestinal microflora and influences the intestinal ecosystem, not only in the composition of the microbiota, but also in the antibiotic resistance profiles and enzymatic potentials of the indigenous microbes. Nitrofurantoin, 1-[(5-nitrofurfurylidene)amino]hydantoin, is a synthetic antibacterial agent, which is effective against most common Gram-negative and Gram-positive urinary tract pathogenic bacteria. Nitrofurantoin-resistant mutants of nitroreductase-producing *Clostridium* species from the human intestinal microflora were selected. The resistant strains metabolized the nitrofurantoin and converted this drug to metabolites without antibacterial activity, as was shown by a bioassay with a nitrofurantoin-sensitive bacterium.

New approaches have been used in the Division of Microbiology to demonstrate that antimicrobial treatments have major impacts on bacterial composition of colonic microbial ecosystems by evaluating the PCR profiles and enzymatic activities of microbial populations before and after antimicrobial treatments. Changes in the total microbial population and in the bacterial sub-populations that are involved in the impairment of intestinal mucosa, metabolism, and enterohepatic circulation of different drugs and food additives were observed after the use of different antimicrobial treatments. A fluoroquinolone antimicrobial agent had more drastic effects than other antimicrobial agents on the bacterial population. In one ulcerative colitis patient, the clinical response to antibacterial therapy was consistent with the decreased bacterial enzymatic activities and changes in the microbial populations as detected by PCR, reiterating that intestinal bacteria and their products play important roles in ulcerative colitis.

The mycotoxin beauvericin, which is one of several toxins produced in corn by fungi of the genus *Fusarium*, has both antimicrobial and insecticidal activities. NCTR micro-biologists (E06922.01) have recently shown that beauvericin inhibits the growth *in vitro* of the Gram-positive anaerobic intestinal bacteria *Bifidobacterium adolescentis*, *Clostridium perfringens*, *Eubacterium biforme*, *Peptostreptococcus anaerobius*, and *P. productus*.

4. Biodegradation assessments of priority pollutants and antibiotics used in aquaculture and their impact on the development of resistance in bacteria.

Bioremediation principles, i.e., the use of microorganisms to degrade pollutants under controlled conditions to an innocuous state or to levels below concentration limits established by regulatory authorities, offers great promise for accelerated removal of chemical pollutants in the environment. A drug registration package must contain data that demonstrates that the proposed substance is efficacious against target pathogens, safe for human use and safe for the environment.

A project (E06901.01) was developed in the laboratory in collaboration with the regulatory scientists of the CVM to evaluate the environmental impact of antibiotics and feed additives used in fish farming systems. Antibiotics are used extensively around the world for control of fish diseases (*vibrioses*) in aquaculture. Currently, the antibiotic erythromycin is under FDA's review for approval for use in salmon and trout culture, specifically for control of bacterial kidney disease. Since aquaculture wastewater and sediment are discharged into the environment, there is concern over the potential detrimental effects on the environment and public health. CVM needs environmental impact and biological activity data on erythromycin for its approval.

Upon reviewing the literature, scientists in the laboratory found that few studies have reported on the environmental fate of erythromycin used in aquaculture. Considering the lack of available information on the environmental impact of erythromycin, the first and foremost challenge was to develop a sensitive bioassay procedure. NCTR was successful in developing a sensitive bioassay procedure to determine biological activity

of erythromycin in aquaculture and environmental samples. This technique is suitable for testing water from marine and aquaculture environments, as well as extracts of a variety of environmental sediments. Separate studies using molecular methods to identify the bacterial species involved have shown that the isolated strain is a new species.

Scientists also studied the behavior of erythromycin under a variety of physicochemical and environmental conditions and found that a variety of microorganisms native to the aquaculture environment were responsible for biodegradation of erythromycin and that a host of metabolites produced lack antimicrobial activity.

Low water solubility is one of the key characteristics of a class of compounds known as the polycyclic aromatic hydrocarbons (PAHs). The PAHs tend to remain in the environment, in part, because the low water solubility limits the microbial degradation of these compounds. The persistence of these chemicals in the environment is a concern because PAHs exhibit toxic, mutagenic and/or carcinogenic effects; they are, therefore, on the U.S. EPA priority pollutant list. Humans can be exposed to PAHs via several routes, including the ingestion of environmentally contaminated foods. For example, fish and shellfish can take up significant levels of PAHs from contaminated sediments; the PAHs are then passed on to humans via consumption of the tainted fish and shellfish. Several strains of bacteria that are capable of degrading some of the more toxic and carcinogenic PAHs, such as pyrene and benz[a]anthracene, have been isolated. The new isolates include members of the genus *Mycobacterium* and others that do not fit into the current classification scheme. The latter probably represent new species and possibly new genera. The isolation of an unidentified bacterium capable of using the very recalcitrant 1,2-benz[a]anthracene as a sole source of carbon and energy is a major breakthrough in the field of PAH degradation. It is anticipated that this organism will prove extremely useful for determining the degradation pathways of higher molecular weight PAHs and has great potential for bioremediation.

Poor understanding of the mechanisms by which bacteria degrade polycyclic aromatic hydrocarbons at the biochemical and molecular level make it difficult to apply bioremediation for the successful removal of these recalcitrant compounds from the contaminated sites. In order to supply the needed information for full understanding of PAH degradation by bacteria for applications in the environmental field, including the development of bacterial strains possessing a superior ability to oxidize many different PAHs, extensive research has been performed to determine the molecular basis for PAH degradation by *Sphingomonas yanoikuyae B1* and *Mycobacterium sp*. *PYR-1* (E06999). *S. yanoikuyae B1* and *Mycobacterium PYR-1* are versatile in their abilities to degrade a number of PAHs. The present work shows that the genes required for the degradation of m-xylene, biphenyl, and naphthalene also give *S. yanoikuyae B1* the ability to metabolize higher-molecular-weight aromatics such as anthracene and phenanthrene. Clones were isolated from a genomic library constructed with the total DNA from *Mycobacterium sp. PYR-1* and their molecular characterization is in progress.

Research in the Division of Microbiology has shown that an environmentally isolated nonpathogenic *Mycobacterium sp*. has susceptibility to isoniazid (an antituberculosis drug) that is similar to that of clinical isolates of *M. tuberculosis* with resistance to isoniazid. Also, like *M. tuberculosis*, it has a catalase-peroxidase that activates isoniazid. By purifying the enzyme from this bacterium and comparing it with that of *M. tuberculosis*, it was shown that *Mycobacterium sp*. could be used as a model to study the target site and the mechanism of drug-resistance development in pathogenic *Mycobacterium spp*.

Nitroreductases are currently being investigated for use as site-specific activators of nitro prodrugs in cancer chemotherapy. *Mycobacterium sp. PYR-1* could be a good source of the enzyme for this purpose (E06959.01). A nitroreductase from *Mycobacterium sp*., which reduces different nitro compounds, including nitro antimicrobial agents, has been purified to homogeneity. The amino acid sequence from the N-terminal end of the nitroreductase from this bacterium and a portion of the internal sequence of the enzyme were shown to be identical to the corresponding sequence of the lipoamide dehydrogenases of *M. tuberculosis* and *M. leprae*.

5. Development of alternative methods for toxicity testing of drugs using microorganisms.

Because of the high costs of animal maintenance and the need to reduce animal use, alternatives or supplementary systems for animal drug metabolism are in high demand. The advantages of a microbial system as a complementary *in vitro* model for drug metabolism are low cost, ease of handling, scale-up capability, and a potential to reduce use of animals. Filamentous fungi have shown the ability to metabolize drugs in a manner similar to that in mammals and are, therefore, potential models for mammalian drug metabolism. The goal in FY98 was to investigate further the potential of the fungal model system to produce a broad spectrum of mammalian drug metabolites and to predict mammalian drug metabolic pathways (E06942.01).

Protriptyline is a tricyclic antidepressant drug that has extensive therapeutic use. The potential of various fungi to metabolize protriptyline was studied in order to show similarities between mammalian and microbial metabolism. Among 27 fungi and yeast species screened, *Fusarium oxysporum var. pini 2380* metabolized 97% of the protriptyline added. Several other fungi screened gave significant metabolism of protriptyline: *Cunninghamella echinulata ATCC 42616* (67%), *Cunninghamella elegans ATCC 9245* (17%), *Cunninghamella elegans ATCC 36112* (22%), *Cunninghamella phaeospora ATCC 22110* (50%), *Fusarium moniliforme MRC-826* (33%), and *Fusarium solani 3179 (*12%). Metabolites produced by each organism were isolated by high-pressure liquid chromatography (HPLC) and identified by nuclear magnetic resonance (NMR) and mass spectrometry. The metabolites identified were 2-hydroxyprotriptyline, N-desmethylprotriptyline, N-acetylprotriptyline, N-acetoxyprotriptyline, 14-oxo-N-des-methylprotriptyline, 2-hydroxy-acetoxyprotriptyline, and N-desmethyl-14-protrip-tylinoic acid. *Fusarium oxysporum var. pini* produced phase I and phase II metabolites and thus is a suitable microbial model for protriptyline metabolism.

Fungi show two different principal metabolic patterns in the biotransformation of diazaarenes, leading either to oxidation of an aza nitrogen or to oxidation of a carbon atom ortho to an aza nitrogen. Phthalazine, a diazaarene with pharmacological relevance because it induces aldehyde oxidase and xanthine oxidase activity, is slightly toxic to aquatic microorganisms and moderately toxic to plants. It is produced during the metabolism of the antihypertensive drug hydralazine and is also a component of certain specialized paper products. *Fusarium moniliforme* oxidized phthalazine to 1(2H)-phthalazinone, which is a metabolite previously found in mammalian systems, but *Cunninghamella elegans* oxidized it to a novel metabolite not produced by mammals, phthalazine N-oxide. Cinnoline, a toxic diazaarene found in diesel engine exhaust, is oxidized by mammals to 4(1H)-cinnolinone, but *C. elegans* and *Aspergillus niger* instead oxidized cinnoline to mixtures of two isomeric N-oxides, cinnoline 2-oxide and cinnoline 1-oxide. The identification of these metabolites was confirmed by HPLC, UV/visible spectrophotometry, mass spectrometry, and NMR spectrometry.

The fungal biotransformation of 6-nitrochrysene and 2-nitrofluorene, two mutagenic and carcinogenic nitro-PAHs that are widespread environmental contaminants, was studied. 6-Nitrochrysene was transformed by *Cunninghamella elegans* to two metabolites that were identified by HPLC, UV/visible spectrophotometry, mass spectrometry, and NMR spectrometry as two isomeric sulfate conjugates that appear to be produced as detoxification products. 2-Nitrofluorene was transformed by *Phanerochaete chrysosporium* to 2-nitro-9-fluorenol and 2-nitro-9-fluorenone, both of which are mutagenic and carcinogenic and, therefore, cannot be considered detoxification products.

The ability of bacteria to biotransform the heterocyclic rings of nitrogen-containing aromatic compounds has been demonstrated. These compounds are found in tobacco smoke, fish from polluted waters, and therapeutic coal-tar preparations. *Streptomyces viridosporus* oxidized isoquinoline, phenanthridine, phthalazine, and quinoxaline, each at a carbon atom ortho to an aza nitrogen, and it oxidized quinazoline at the carbon atoms ortho to both of the two aza nitrogens. HPLC, UV/visible spectrophotometry, mass spectrometry, and NMR spectrometry were used to identify the metabolites. *S. virIdo-sporus* further metabolized the product from quinoxaline, 2(1H)-quinoxalinone, by means of a novel N-methyltransferase to form 1-methyl-2(1H)-quinoxalinone.

FY99 Goals

1. Determine the role of intestinal microflora in the activation or detoxification of xenobiotics.

Research on the role of gut microflora in human carcinogenesis is an important FDA need since a high proportion of human cancer is caused by environmental factors, and diet may be particularly important.

Since the bacterial flora are in a uniquely favorable position to mediate the interaction between the gut contents and the host, it would be surprising if bacteria were not implicated in human carcinogenesis. Therefore, the focus of this research component is: 1) to use existing models for determining the contribution of the gut microflora to foreign-compound metabolism in humans and laboratory animals; 2) to relate bacterial metabolism to toxic events occurring in mammals; 3) to consider the interrelationships of bacterial and mammalian metabolic pathways; 4) to determine the effect of dietary components on the composition of the microflora in the human gastrointestinal tract; and 5) to determine the genes involved in the metabolism and activation of pharmaceutical azo- and nitro-compounds, food additives and endocrine disruptors.

Research goals for this sub-program are: 1) to delineate the metabolic potential of intestinal microorganisms and the enzyme mechanisms by which they transform drugs, azo dyes, food additives, endocrine disruptors, and triphenylmethane dyes; 2) to develop additional models for assessing the risk to human health posed by exposure to synthetic and naturally occurring chemicals; and 3) to determine the pharmacological and toxicological effects of the metabolism of chemicals such as food additives, azo compounds used as protective coatings for drug delivery and pro-drug azo compounds, and antimicrobial compounds on the intestinal microflora.

2. Use microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals.

In recent years, interest has turned to the development of alternative systems for decreasing the use of animals in laboratory studies. Eukaryotic microorganisms can be practical substitutes for the rodents and other mammals currently used in many studies on the metabolism of pharmaceutical drugs and xenobiotic compounds. The advantages of microbial systems include: 1) ease of experimental manipulation; 2) ease of scale-up for production of metabolites that other investigators can use for structure elucidation, biological evaluation, and analytical standards; 3) lower cost; and 4) reduction of the use of laboratory animals. The focus of this research component is to develop alternative methods for studying the metabolism of pharmaceutical drugs and other chemicals of interest to the FDA. Substantial research has already shown that certain fungi have the capacity to produce pharmaceutical drug metabolites that are the same as, or similar to, those produced by higher eukaryotes. The current goal is to develop a refined fungal system that more closely mimics the metabolism of pharmaceutical drugs in humans and to use the refined system for studying possible drug-drug interactions and for producing metabolites for further toxicological studies. This research will provide more accurate risk assessments and a better understanding of the mechanisms of metabolism of new drugs and the potential for drug-drug interactions.

3. Develop environmental biotechnology.

FDA's pre-market review considers potential environmental impact during the entire life-cycle of a regulated product, including its manufacture, use and disposal. Under the FDA's environmental regulations, the industry sponsor of an application or petition may be required to prepare an environmental assessment of the proposed action. To support the assessment, appropriate testing of the environmental fate and effects of chemicals entering the environment may be required. The need for testing is determined by evaluation of the potential environmental exposure and the toxicity information available for a given chemical.

Due to the high cost associated with trapping, incinerating or physically removing toxic chemicals from the environment, there has been an increased interest in the use of microorganisms for the biological decontamination and detoxification of hazardous waste sites. Because the environmental risk assessment and management of potentially hazardous chemicals requires information on their occurrence, toxicity, bioavailability and persistence in the environment, the program has developed multi-component environmental microcosms. These microcosms are useful for determining the rate and pathways for the environmental biodegradation of xenobiotics. The focus of this research is to isolate microorganisms that can degrade, detoxify, or accumulate hazardous chemicals and to determine the potential for their use in the bioremediation of toxic waste sites. This methodology will be used for several FDA-related research problems combined with the fate of antibiotics in the environment.

4. Develop methods for detection of contaminants.

Foodborne bacterial pathogens have been detected in contaminated foods using molecular genetic methods. Effective and sensitive methods are needed to detect contamination in foods to determine if the levels of contamination pose a public health risk. Polymerase Chain Reaction (PCR)-based methods have the potential for revealing the presence of pathogenic microorganisms in foods in a few hours while current methods require two days or longer. Rapid detection and identification of bacteria are important not only for food safety, but also for the study of the significance of the species on both *in vitro* and *in vivo* metabolic activation and detoxification of chemical toxicants and drugs and for the diagnosis of the diseases caused by these species. Development of better *in vitro* methods for rapid detection of bacterial pathogens and toxins will provide the FDA with analyses critically needed for assurance of food safety and enforcement of regulatory compliance.

5. Continue microbiological surveillance and diagnostic support of research.

Laboratory animals are susceptible to a wide variety of bacterial, viral and parasitic infections, resulting in an altered animal model that consequently affects research and testing by introducing variables that confound results. Routine screening for various infectious diseases assures reliable animal models and prevents costly, time-consuming

delays of research that could affect FDA regulatory decisions. Studies utilizing animals are dependent on healthy test animals; therefore, it is NCTR's responsibility to maintain the best microbiological diagnostic laboratory possible. The investigators and the FDA should be able to depend upon NCTR to support their efforts. Research goals for this sub-program are: 1) establishing and maintaining pathogen-free animals; 2) culturing and identifying microbial contaminants for other projects and programs within the NCTR and other FDA centers; and 3) developing and testing new methods in diagnostic microbiology for other FDA centers.

Significance to the FDA

The Division of Microbiology seeks to continue and expand its scientific exchange and collaborative studies with colleagues at other FDA centers and field laboratories to anticipate their research needs and provide data to support regulatory activities of the Agency.

These studies include: 1) metabolism and toxicological effects of food additives, antimicrobials and macronutrients on the intestinal microflora; 2) microbial production of metabolites of toxicological and pharmaceutical interest; 3) environmental fate and effects of aquaculture chemicals and other priority pollutants; 4) tuberculocidal disinfectant testing; 5) detection of foodborne biological hazards; 6) rapid and accurate detection methods for pathogens and toxins; and 7) microbial surveillance of experimental animals.

Many of the techniques currently in use within the microbiology research area are of value to other FDA centers and field laboratories. As communication and discussion of mutual research interests between NCTR staff and other FDA scientists increases, many new projects at the forefront of applied microbiology research will be developed. The laboratory's vision is to strive for scientific excellence and to strengthen the relevance of its research with the mission of the Food and Drug Administration. It will continue to maintain a world-class research program to solve current issues that face the FDA in the next millennium, so the Agency can make sound, science-based regulatory decisions on microbiological issues.

MOLECULAR EPIDEMIOLOGY

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Development of rapid genotyping methods for a variety of susceptibility markers in humans.

Introduction

The strategic goals of the Division are: 1) the identification of genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy; 2) the conduct of epidemiological studies for post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices; 3) human exposure biomonitoring and DNA adduct detection; 4) the extrapolation of the results of animal bioassays and of mechanistic studies to humans; and 5) the development and validation of "DNA Microarray Technology" for human diagnostics.

The intent is to better understand the mechanisms of human carcinogenesis; to provide an estimation of human exposure to direct and indirect-acting carcinogens; to assess the importance of inter-individual differences in carcinogen and drug bioactivation, detoxification, or induced changes in gene expression; and to suggest intervention strategies for human cancer prevention. Accordingly, research has provided new knowledge on the identification of subpopulations that are not only more susceptible to chemical carcinogens, but also those that are likely to experience adverse drug reactions or decreased therapeutic drug efficacy. Research has been focused on the foodborne heterocyclic amines, aromatic amines, and polycyclic aromatic hydrocarbons, and on widely used drugs including selected benzodiazepines, antihistamines, drugs inducing peroxisomal proliferation or oxidative stress, estrogens, anti-estrogens and endocrine disruptors, as well as on tobacco usage. Projects on the etiology of human cancers of the colon/rectum, pancreas, larynx, breast, ovary, prostate, lung, urinary bladder, bone marrow, and esophagus are ongoing.

Studies to identify genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy:

1. Metabolic polymorphisms, DNA repair, and individual cancer susceptibility.

    a) Genetic and epigenetic regulation of cytochrome P450 1A2.
    b) Polymorphisms of cytochrome P450 1B1 and tissue-dependent expression.
    c) Polymorphisms of cytochromes P450 2A6 and P450 2E1.
    d) Polymorphisms of phenol and estrogen sulfotransferases.

e) Polymorphisms of glutathione S-transferases A1, A2, and P1.
f) Inter-individual variation in DNA repair capacity.
g) Substrate specificity and activity of COX-1 and COX-2 toward metabolic activation of foodborne carcinogens.
h) Gender-specific variation in drug metabolism.

2. Chemoprevention.

a) Modulation of expression of multi-drug resistance genes.
b) Coffee and tea effects on cytochrome P450 1A2, glutathione S-transferases, and N-acetyltransferases.
c) Effects of tea extracts on expression of genes cytochrome P450 1A2, H-*ras*, and the normal epithelial cell specific gene (NES1).
d) DNA methylation, DNA methyltransferases, and homocysteine toxicity.

Epidemiology and post-market surveillance for chemical toxicants found in foods, drugs, cosmetics, and medical devices:

3. Etiology of human colorectal cancer: role of dietary heterocyclic amines.

a) Etiology of human breast and prostate cancers in African-Americans.
b) Etiology of human pancreatic cancer: role of carcinogen & drug exposures, chronic pancreatitis, and dietary imbalance.

Human exposure biomonitoring and DNA adduct detection:

4. Biomarkers of exposure and susceptibility for breast, prostate, ovarian, laryngeal, esophageal, lung, colon, and urinary bladder cancers.

Extrapolation of the results of animal bioassays and of mechanistic studies to humans:

5. Evaluation of the neonatal mouse bioassay as an alternative bioassay for selected benzodiazepines, antihistamines, chloral hydrate, drugs inducing peroxisomal proliferation or oxidative stress, synthetic and natural estrogens, and endocrine disruptors, including chlorinated hydrocarbon pesticides and dinitroaniline herbicides.

International efforts in molecular epidemiology and biotechnology:

6. Organization of the Molecular Epidemiology Group of the American Association for Cancer Research.

7. Development and validation of "DNA Microarray Technology" for assessing individual risk for cancer susceptibility and recurrence, adverse drug reactions, and therapeutic drug efficacy.

FY98 Accomplishments

During 1998, Division studies on genetic polymorphisms were focused on the bioactivation and detoxification of the foodborne heterocyclic amines, which have been of increasing public health concern to FDA.

Studies to identify genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy:

1. Metabolic polymorphisms, DNA repair, and individual cancer susceptibility.

Genetic and epigenetic regulation of *CYP1A2*. Using animal models, human tissues, and molecular biomarkers in epidemiological studies, the bioactivation of heterocyclic amines to colon carcinogens in humans was previously found to involve N-oxidation followed by O-acetylation to form the *N*-acetoxy arylamine that binds to DNA to form carcinogen-DNA adducts. These steps are catalyzed by the hepatic enzymes, *CYP1A2* and *NAT2*, respectively, which NCTR and others have shown to be expressed polymorphically in humans. The Division has previously identified four variant alleles in the *CYP1A2* gene, and they now have evidence that one of these is a common genetic variant in human populations and is associated with *CYP1A2* inducibility. The sequence change involves GGGCAC→GGGCCC, which they have characterized as a negative regulatory element. A simple restriction fragment length polymorphism (RFLP) genotyping assay is currently being used to determine the frequency of the four allelic variants in human populations. The Division has also investigated the regulation of *CYP1A2* (and *CYP1A1*) gene expression through epigenetic (methylation) mechanisms. Preliminary data have demonstrated inter-individual differences in constitutive expression and enzyme activity of *CYP1A2* in human liver tissue grouped according to gender, age and smoking status. Initial results on DNA methylation profiles of one of the *CYP1A2* gene promoter regions (-3030 to -2490), which contains one CCGG site next to an AP-1 site, indicates that it is hypermethylated (C^mCGG) in liver tissue from older female smokers. This site, in both the young and old male smokers, is hypomethylated (CCGG). Further studies are being conducted to correlate the methylation profiles with gene expression and enzyme activity. Studies are also being conducted to examine the overall methylation status of the *CYP1A2* gene. It is believed that these findings will have a major impact not only on cancer susceptibility, but also on therapeutic drug efficacy and hormonal interactions, since *CYP1A2* is a major inducible enzyme metabolizing many drugs and estrogens.

Polymorphisms of cytochrome P450 1B1 and tissue-dependent expression. Another benefit of this type of work relates to proper prescription of hormonally active therapeutic drugs. The recently discovered cytochrome P450 1B1 (*CYP1B1*), because of its substrate specificity, has been hypothesized to play a role in breast, ovarian, and prostate cancer, as it metabolizes estrogens, testosterone, and certain carcinogenic aromatic amines and polycyclic hydrocarbons. The Division has recently discovered a genetic variant of *CYP1B1* and has developed a method for rapid genotyping of human

populations. They have now found that this variant, in which leucine is replaced by valine in the protein, appears to be a high activity allele and is significantly over-represented in patients with prostate cancer. This variant is also being examined as a risk factor in the breast cancer case-control study that is now underway. It is expected that this novel finding will provide important information on the etiology of these cancers and, thus, the efficacy and safe levels of hormonally active drugs.

Polymorphism of cytochrome P450 2E1. In collaboration with Professor Dong-Xin Lin, a visiting scientist at NCTR and now Professor and Director of the Division of Cancer Etiology and Carcinogenesis at the National Cancer Institute in Beijing, the Division has provided the first evidence that a genetic polymorphism in the nitrosamine-metabolizing enzyme, cytochrome P450 2E1 (*CYP2E1*), is a strong risk factor for esophageal cancer in China (Linxian County), where foodborne nitrosamines known to be bioactivated by *CYP2E1* have been strongly implicated in the etiology of this cancer. These data indicate that food-borne nitrosamines are probable human carcinogens and support the continued monitoring of our food supply for carcinogenic nitrosamines by FDA/ORA.

Polymorphism of phenol sulfotransferase. Sulfation is an important pathway in the metabolism of many drugs, xenobiotics, and endogenous steroid hormones and human tissues express five cytoplasmic sulfotransferase (*SULT*) enzymes. The phenol sulfotransferase, *SULT1A1*, has been studied in relation to colorectal cancer. This detoxifying enzyme, which conjugates drug metabolites to facilitate renal clearance and urinary excretion, also acts on *N*-hydroxy aromatic amines, rapidly converting them to their phenolic sulfates and decreasing their bioavailability for bioactivation by *NAT2*. They have also shown this same enzyme to be present in human platelets and have developed and validated a rapid phenotyping assay for application to epidemiological studies in colorectal and urinary bladder cancer.

Polymorphisms of GSTs A1, A2, and P1. The alpha class GSTs are major detoxification enzymes for a wide variety of carcinogens in the liver. These are subject to differential expression in different tissues and are known to be inducible by fruits and vegetables, known to be consistent protective factors against human cancers. The basis of the apparent polymorphic expression of GST A1 and A2 (alpha class) has been investigated at the molecular level by DNA sequencing of the 5'-regulatory regions of DNA isolated from livers of individuals that express high levels of GSTA1 both relative to GST A2 and in absolute terms, and vice versa. Although a linked polymorphism was found at three positions, -52, -69, and -567 bp in the GST A1 regulatory sequence, i.e., partly within the purported regulatory region, these changes did not correlate with differences in expression. Thus, the basis for differential GST A1 and GST A2 expression may simply be a conse-quence of differential induction due to dietary components. Refinement of GST phenotype analysis has shown that alpha class GST expression in the pancreas is complex with at least six forms, A1, A2, A3, A4, A5 and A6. The A4 protein has not previously been identified at the protein level, A5 appears to be a form belonging to a group of three GST A2-like genes. GST A5 appears to show a polymorphism possibly with identical regulatory sequence to that of GST A2, although GST A2 and GST A5 are thought to be the products of separate genes. GST A4 is of interest because of its predicted high activity towards the toxic lipid peroxidation product,

2-hydroxy-4-nonenal. GST A6 does not correspond to the product of any known human alpha class gene. As all of the alpha class genes show GSH-dependent peroxidase activity, their expression and regulation are of particular relevance to pancreatitis and colon cancer which share the risk factors of a high energy diet, putatively associated with lipid peroxidation. The Division's results in the pancreas suggest that GST A4 and GST A6 are highly inducible, with inter-individual variation in expression varying by 180- and 100-fold, respectively.

Inter-individual variation of GST phenotype. Inter-individual variation of GST phenotype has been investigated in lung and pancreas. Results show that a 10 to 30-fold variation appears to be within the normal range of constitutive expression. Establishment of range of constitutive expression puts into context the differences of enzyme activity that result from genetic polymorphisms. In the pancreas, significant correlations were found to exist between expression of GST P and GST M3 and, in GST M1-positive samples, between GST P1 and GST M1. While the basis for this is unknown, such correlations may simplify the analysis of GST expression and provide a greater understanding of the regulation of these critical detoxification enzymes.

Gender-specific variation in drug metabolism and DNA repair. In 1998, funding was awarded through the FDA Office of Women's Health to support the recruitment of 160 individuals to participate in a gender- and age-related study of drug metabolism. This study is focused on the role of hormones, oral contraceptive usage and hormone replacement therapy on *CYP1A2* activity. A successful recruitment strategy is in place with 25% of the study participants recruited since September 1998. With Institutional Review Board approval and active banking protocols in place, sample analysis began in early November,1998.

Detoxification of N-hydroxy heterocyclic and aromatic amines by enzymatic reduction to the parent amine. The metabolic activation pathways associated with carcinogenic aromatic amines have long been known to involve *N*-oxidation, catalyzed primarily by cytochrome P450 1A2 (*CYP1A2*), and subsequent *O*-esterification, often catalyzed by acetyltransferases (*NATs*) and sulfotransferases (*SULTs*). In current studies, the Division has found a new enzymatic mechanism of carcinogen detoxification: a microsomal NADH-dependent reductase that rapidly converts the *N*-hydroxy arylamine back to the parent amine. This investigation arose as a consequence of *in vitro* studies with the heterocyclic amine carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP). Unlike Aroclor-induced rat liver primary hepatocytes, uninduced rat hepatocytes incubated with PhIP, produced no *N*-oxidized metabolites and incubation with N-OH-PhIP showed nearly complete conversion to the parent amine. This led them to hypothesize that a cytochrome b_5-dependent pathway, first reported some 25 years ago and studied in pig and rat liver microsomes, was involved. They have now characterized this NADH-dependent and oxygen-insensitive *N*-hydroxy (N-OH) amine reductase activity in rat and human liver microsomes and found it to be comparable to that reported previously for N-OH-alkylamines. In human liver microsomes, the following carcinogenic N-OH-arylamines and N-OH heterocyclic amines were rapidly reduced: N-OH-PhIP, N-OH-2-amino-carboline, N-OH-4-amino-biphenyl, N-OH-2-naphthylamine, N-OH-2-aminofluorene, and N-OH-4,4'-methylene*bis*-(2-chloroaniline).

Individual human liver microsomal samples (n = 16) exhibited activity levels over a broad range of 0.2 to 5.4 nmol/min/mg protein. In addition, intact rat liver primary hepatocytes and intact human HepG2 cells efficiently reduce N-OH-PhIP to PhIP with no cofactors added. This previously unrecognized detoxification pathway may limit the bioavailability of carcinogenic N-OH heterocyclic and aromatic amines for further activation, DNA adduct formation, and carcinogenesis.

2. Chemoprevention.

Modulation of expression of multi-drug resistance genes. Acquired resistance to drugs such as cisplatin or its less-cytotoxic analog, carboplatin, is a characteristic of several tumors cells, especially human tumor bladder cells and is often associated with the multi-drug resistance (MDR) phenotype. The development of MDR in cells during chemical carcinogenesis is also associated with changes in the expression of several phase II drug-metabolizing enzymes. The active antineoplastic agent, cisplatin, induces a complex toxicological response in tumor cells, including DNA adduct formation and intrastrand cross-linkage that may lead to cell death or acquired drug resistance. Since elevated expression of phase II-linked detoxification enzymes is often implicated in differential susceptibility and acquired drug resistance, they have examined the effect of heterologous expression of *SULT*s on the mutational profile induced by cisplatin at the hypoxanthine guanosine phosphoribosyl transferase (HGPRT) locus of transfected CHO cells. Currently, the Division has transfected human urinary tract transitional cancer cell lines 253J and 647V with *SULT1A1* and *SULT1C1*. These transfected cells are being characterized for *SULT* and *mdr-1* gene expression, and mutational profiles after anguidine and cisplatin treatment. The use of this *in vitro* model will also aid in understanding the role of these enzymes and their effects on cancer chemotherapeutic efficacy.

Coffee and tea effects on *CYP1A2,* GSTs, and *NATs*. Since colorectal cancer is one of the most prevalent cancers in non-smokers in the U.S., appropriate intervention strategies and concomitant public health recommendations are of paramount importance. To determine the potential for dietary intervention in modulating the carcinogenicity of the heterocyclic amines, the effect of a variety of treatment regimens on the bioactivation and detoxification pathways has been examined. Of these, consumption of black tea and the coffee constituents kahweol and cafestol were most effective. Their mechanisms of action were studied and shown to involve the potent inhibition of *CYP1A2* by black tea, induction of alpha-class GSTs and down-regulation of *NATs* by kahweol/cafestol. The latter finding is the first evidence that *NAT2*, the oldest known human drug polymorphism, can also be regulated by dietary factors and could explain putative idiosyncratic adverse drug reactions in humans.

DNA methylation, DNA methyltransferases, and homocysteine toxicity. Additional studies on chemoprevention in the Division have included the modulation of nutrients as well as of non-nutritive dietary components. They have extended findings that calorie restriction markedly protected against changes in gene expression (through gene methylation) by maintaining SAM levels and preventing the formation of preneoplastic lesions produced in the livers of rats treated with aflatoxin B_1 together with feeding a

methyl-deficient diet. They have published findings indicating that the expression of the "viable yellow" gene, which is up-regulated by a hypomethylated viral promoter insert, can be partially prevented by feeding a diet rich in betaine, choline, and folic acid. The "viable yellow" phenotype is associated with an increased susceptibility to carcinogenesis. Dietary modulation can thus foster normal embryonal development even *in utero*. These results support FDA's recommendation to increase the folic acid content of the American diet.

Of more direct bearing to carcinogenesis, however, is that dietary intervention can prevent cancer induction and other pathologic effects even after a genetic alteration has occurred. Interestingly, the Division has preliminary data that have not shown any significant difference in SAM levels in human liver tissues grouped according to gender, age or smoking status. However, a significant increase in DNA methyltransferase activity was noted in liver tissue from young female smokers. An elevated level of this enzyme in tumors and in preneoplastic tissue constitutes one of the most ubiquitous occurrences in cancer. They have recently described an abnormal methylating activity of a DNA methyltransferase obtained from tumors occurring in methyl-deficient rats. A similar enzymatic activity was not seen in normal liver. This observation raises the prospect that the abnormal form of the enzyme may serve as an endogenous carcinogen. They are thus attempting to extend this observation to human tumors and to improve the analytical methodology required.

In this regard, new findings from the Division is expected to increase the validity of extrapolations between animal models and humans. The development of routine assays for SAM, S-adenosylhomocysteine (SAH), homocysteine, and 5-methyldeoxycytidine has encouraged several groups to seek collaborations on the possible causative role of abnormal methyl metabolism on cancer formation in humans. Only recently has a similar association been found in humans. Populations that are at particularly high risk of cancer development, because of diet, carcinogen exposure, or genetic abnormalities, could thus be screened for SAM/SAH (folate) insufficiency. In animals and in cells in culture, such a causal relation has long been known.

Epidemiology and post-market surveillance for chemical toxicants found in foods, drugs, cosmetics and medical devices.

3. Etiology of human colorectal cancer: role of dietary heterocyclic amines.

In 1998, the Division continued its case-control study of colorectal cancer with UAMS and have enrolled about 200 cancer cases and 300 control subjects in the study. *CYP1A2* and *NAT2* genotyping and phenotyping is ongoing and a novel questionnaire, which was developed and validated in collaboration with the NCI, is now being used to more accurately estimate exposure to foodborne carcinogens. Furthermore, with their development of a rapid phenotyping assay for *SULT1A1*, they have recently reported that the high-activity phenotype is protective for colorectal cancer, with 60% prevalence among controls and only 40% among cancer cases.

This research has shown that, consistent with the proposed metabolic activation pathway for heterocyclic amine carcinogens, subjects at greatest risk for colorectal cancer or non-familial polyps are those who possess both the rapid *NAT2* genotype/ phenotype, the rapid *CYP1A2* and slow *SULT1A1* phenotypes, and who are exposed to high dietary levels of carcinogenic heterocyclic amines. Moreover, a logistic regression model that included both metabolic genotypes/phenotypes and consumption of well-done red meat suggests that, in terms of attributable risk, these susceptibility factors together with foodborne heterocyclic amine carcinogen intake may account for over half of the sporadic colorectal tumor incidence observed in the U.S.

4. Etiology of human breast and prostate cancers in African-Americans.

During the last two years, extramural funding was received to support large case-control studies of breast and prostate cancer. In the last year, recruiters who are breast and prostate cancer survivors have been trained and are actively recruiting new patients. The Division has successfully enrolled over 100 women into the breast cancer study, and recruitment has begun for the prostate cancer study. They have established collaborative relationships with physicians at Jefferson Regional Medical Center in Pine Bluff, Arkansas, and at Methodist Cancer Center in Memphis, Tennessee. Patients will be identified from these hospitals to increase enrollment of African-American men and women living in the Mississippi Delta region.

Because of the racial disparities in breast and prostate cancer incidence, and the likelihood that diet may play a profound role in the etiology of both diseases, the Division conducted a survey of dietary habits of rural African-American men and women in the Mississippi River Delta region in eastern Arkansas. There is little information regarding eating habits of rural African-Americans in the southern United States, and it is questionable if existing food-frequency questionnaires are relevant for these populations. Using focus groups of African-American women, over 60 foods have been identified that are also commonly eaten in this region, but are not included on standard Food Frequency questionnaires in the U.S. Over 200 African-American women have been surveyed to date to establish the extent to which these foods are consumed and if they should be included in studies of diet and cancer among African-Americans. Preliminary analyses indicate that foods frequently eaten that are not usually included on food-frequency questionnaires include dried beans and peas common to the South, cooked with fat, greens cooked with fat, and fried catfish. Furthermore, additional fat is commonly added to most vegetables and gravy is eaten with biscuits. These sources of fat, if not recorded in dietary surveys, could be contributing factors to cancer etiology that have not been previously recognized in African-American populations.

5. Etiology of human pancreatic cancer: role of carcinogen and drug exposures, chronic pancreatitis, and dietary imbalance.

Another project involving the use of molecular biomarkers in a pancreas cancer case-control study is nearly completed with some 300 cases and 400 controls entered into the study. In the interim, the Division has examined human pancreatic tissues for the presence of carcinogen-DNA adducts derived from environmental exposures (tobacco,

food, drugs, etc.) and endogenous DNA adducts derived from oxidative damage and lipid peroxidation. Recently, they have examined human pancreas tissue where cancer risk has been associated with a number of factors suggestive of either exogenous chemical exposure and/or endogenously derived DNA damaging agents. To provide a mechanistic basis for the types of DNA lesions formed in human tissues, we have also focused on the role of gene-environment interactions as a critical determinant in which carcinogens, regardless of source, are bioactivated or detoxified, thus accounting for individual susceptibility to a given exposure. They reported on the levels of hydrophobic aromatic amines (AAs), specifically those derived from 4-aminobiphenyl (ABP), and the DNA adducts associated with oxidative stress in the human pancreas. Using the same DNA, the *NAT*1, GST M1, GST P1, GST T1, and NAD(P)H quinone reductase-1 (NQO_1) genotypes were determined to assess the role of their gene products in modulating adduct levels through their involvement in detoxification of AAs, lipid peroxidation products and redox cycling. These studies indicate that ABP-DNA adducts, malondialdehyde-DNA adducts, and 8-oxo-2'-deoxyguanosine (8-oxo-dG) adducts are present at similar levels. Of the metabolic genotypes examined, the presence of ABP-DNA adducts was strongly associated with the putative slow *NAT1*4/*4* genotype, suggesting a critical role for this pathway in ABP detoxification.

Chronic pancreatitis, which also induces oxidative stress, is considered an important precursor lesion in a number of pancreatic cancer cases and is induced by several drugs and by chronic alcohol use. In particular, nucleoside analogs, such as dideoxy-inosine (ddI) and ddC, currently being used to treat HIV-infected individuals, cause acute pancreatitis. To determine the molecular mechanisms involved in ddI toxicity and in induced pancreatitis, they have utilized an animal model that involves treatment of normal rats treated with ddI at various doses and time periods and subsequent analysis of several biochemical and molecular biological endpoints. These studies have revealed that ddI causes abnormal DNA methylation in pancreatic tissue from animals dosed more than 10 weeks, showing decreased levels of SAM. Pancreatic acinar cells isolated from ddI-treated animals and subcultured for several passages also revealed a remarkable decrease in SAM levels. Cultured pancreatic acinar cells from ddI-treated rats further demonstrated malignant transformation characteristics such as enhanced growth and the activation and hypomethylation of the K-*ras* protooncogene. The increased expression of the *ras* protein, which is a potent inducer of the transcription factor, AP-1, indicates that ddI may exert its adverse effects on the pancreas through epigenetic mechanisms involving changes in the overall methylation status, thus suggesting folate supplementation as a possible intervention strategy.

Diets low in micronutrients, especially antioxidants, have recently been shown to induce pancreatitis in rats, apparently by decreasing cell defenses against free radical-induced damage. The development of their *in vitro* model, culturing pancreatic acinar cells from ddI-treated animals, has allowed the ability to examine micronutrient effects on SAM levels in these cells. These studies demonstrated that folic acid and low concentration of $ZnCl_2$ significantly increased SAM levels in ddI-treated cells. Only high concentrations of vitamin C were shown to increase SAM levels. These studies thus demonstrate the potential use of antioxidants in decreasing the side effects caused by some drugs such as ddI.

Human exposure biomonitoring and DNA adduct detection.

6. Biomarkers of exposure and susceptibility for breast, prostate, ovarian, laryngeal, esophageal, lung, and urinary bladder cancers.

Researchers in the Division have been collaborating with other groups with completed studies of breast cancer to evaluate innovative hypotheses. In analysis of data from a study of breast cancer in western New York, they have evaluated the role of genetic polymorphisms in carcinogen and hormone metabolizing enzymes. Catechol O-methyltransferase (COMT) is involved in metabolism of estradiol, and analyses have indicated that the role of COMT in breast carcinogenesis may vary by menopausal status. While the polymorphism associated with the low activity phenotype increases risk among premenopausal women, there is an inverse relationship with postmenopausal breast cancer. In these same data, they have also observed an effect of the rapid *NAT1* allele on risk of postmenopausal breast cancer among smokers, but not non-smokers, and there was a strong effect when combined with women with the slow *NAT2* genotype. They also evaluated the possible role of meats and fish in breast cancer etiology, and possible modification of risk by *NAT2*. Consumption of meats and those cooked at high temperatures did not increase breast cancer risk, regardless of *NAT2* status. Those in the highest quartile for fish consumption, however, were at reduced breast cancer risk. They have also evaluated the role of a polymorphism in manganese superoxide dismutase involved in protection of mitochondria from oxidative stress, in relation to breast cancer risk. Premenopausal women with the variant alleles were more likely to have breast cancer than those with common alleles. This effect was most striking among women who consumed lower amounts of fruits and vegetables than the median. With this same group, we also evaluated possible gene-environment interactions between genetic polymorphisms in *NAT2* and GST M1 and smoking on risk of recurrent spontaneous abortion. There was a main effect of smoking on risk, but genetic variability did not modify it. However, numbers of women with recurrent spontaneous abortions were small, and limited power may have affected the inability to detect an association.

In their investigation of etiologic factors in breast and prostate cancer, the Division has been striving to identify biomarkers of exposure. In the past year, DNA from exfoliated ductal epithelial cells has been extracted, and bulky aromatic carcinogen-DNA adducts detected. They have established collaborations with researchers at EPA and the University of Guelph. In a pilot study, levels of DNA adducts were significantly associated with mutagenicity of milk specimens. Similarly, studies have been underway to characterize carcinogen-DNA adducts in prostate tissue. Tissues obtained from needle-biopsy procedures have been used for DNA extraction, and aromatic carcinogen-DNA adducts have been detected. Preliminary results indicate that these adducts may be derived from aromatic and heterocyclic amines.

The foodborne phytoestrogens are a diverse group of diphenolic compounds similar to estrogens. They have been characterized as having estrogenic, anti-estrogenic, anti-carcinogenic and carcinogenic potential and, therefore, may pose a health problem for women consuming diets high in phytoestrogen content. Examining long-term effects (six months) of phytoestrogens, after administering at critical periods of development (PND, 1-5) in rats, demonstrated tissue specificity in relationship to adverse effects. This study, which was conducted in both intact and ovariectomized rats, showed that coumestrol and equol activated protooncogenes (*c-myc,* K-*ras,* H-*ras and c-fos*). Diethylstilbestrol (DES) was used as a positive control in all studies. Adverse effects (increased expression of protooncogenes or hypomethylation) were noted in the following tissues: cervix, uterus, ovaries and lungs; however, a possible beneficial effect was noted with pancreatic tissue where hypermethylation of the H-*ras* gene was observed. These studies indicate that both of the above phytoestrogens exhibited differences in protooncogene activation in various tissues and further suggest that phytoestrogens could exhibit both beneficial and adverse effects in tissue in relation to carcinogenic potential.

Extrapolation of the results of animal bioassays and of mechanistic studies to humans.

7. Evaluation of the neonatal mouse bioassay as an alternative bioassay for extrapolation between animal studies and human populations.

These projects have thus far focused primarily on the validation of the neonatal mouse bioassay as an alternative model for identifying genotoxic carcinogens (see also "Accomplishments" under the Division of Biochemical Toxicology). The evaluations of several widely used benzodiazepine and antihistamine drugs, as well as methylphenidate, and chloral hydrate and its metabolites have been completed. Ongoing studies include several drugs inducing peroxisomal proliferation or oxidative stress; synthetic and catechol estrogens; and putative endocrine disruptors, including phytoestrogens; chlorinated hydrocarbon pesticides and dinitroaniline herbicides. These results are being compared to studies being conducted by the National Institute for Environmental Health Sciences (NIEHS) on other alternative rodent bioassays. The compounds selected represent major classes of drugs that are widely used in human populations. A common concern for many of these compounds arises from drug-related increases in the incidence of mouse liver tumors observed in standard two-year carcinogenicity studies. In this regard, the mechanism of tumor induction is unclear and both genotoxic and nongenotoxic processes have been proposed. However, in the neonatal mouse tumorigenicity bioassay, only two doses of the test compound, given to preweanling animals, are required to obtain positive results after 12 months; and, thus far, only genotoxic carcinogens have been shown to be active in this test system. Therefore, it is believed that this bioassay, when combined with relevant mechanistic information in human cells and in human epidemiological studies, will provide a more definitive assessment of the significance of marginal findings in the standard rodent bioassay and will also become a useful supplemental or alternate carcinogenicity screening method for FDA-regulated drugs or drug products.

International efforts in molecular epidemiology and biotechnology.

8. International efforts in public health and molecular epidemiology.

In international activities, Division staff, with help from CFSAN and CVM staff, have recently completed a four-year task, which involved their participation in a panel of experts representing the World Health Organizaiton and several foreign governments and resulted in the publication of new book on "Diet, Nutrition and Cancer: A Global Perspective." This book, which will be distributed to 100,000 health professionals worldwide by the American Institute of Cancer Research/World Cancer Research Fund, is expected to have a major impact on public health policy and strongly supports the importance of FDA research and regulatory efforts in maintaining food safety and promoting good nutrition for decreasing cancer risk.

Finally, this Division has played the lead role in organizing the newly formed Molecular Epidemiology Group of the American Association of Cancer Research. This group will greatly facilitate collaborative relationships and cross-training of researchers from numerous disciplines worldwide, which will impact on understanding the role of substances regulated by the FDA in carcinogenesis as well as to identify susceptible subgroups.

9. Development and validation of DNA microarray technology for human diagnostics.

In 1998, the Division initiated a large collaborative effort with multi-center participation between the M.D. Anderson Cancer Center, the Arizona Cancer Center, NCTR, and Genometrix to validate the use of DNA microarray technology for automation and large-scale genotyping. NCTR has initiated the procurement of a set of DNA standards to be used in the validation of rapid, sensitive and reproducible PCR-based methodologies for near-simultaneous screening of genetic polymorphisms in xenobiotic metabolizing enzymes, DNA repair enzymes, and key cancer susceptibility genes for large population studies. Based on previous work in this and other groups, a “Risk-Tox Chip” has been designed and funding through a three-year CRADA, with additional funding from NIEHS, has been obtained for validation studies. To date, a “mini” platform with ten alleles is built and is undergoing validation at Genometrix with a series of genotyped DNA samples from NCTR. Work on the development and fabrication of the microarray chip or “Risk-Tox Chip” for the analysis of genetic polymorphisms that affect individual cancer or adverse drug risk has been initiated at Genometrix and validation of the “risk chip” by comparative analyses with standardized methodologies has begun at NCTR and is at 20% completion. Automation of methodologies for large population risk assessment using the “Risk-Tox Chip” in a robotic workstation is near completion (early 1999).

FY99 Plans and Goals

During 1999, the Division will continue with the projects described under the section, "1998 Accomplishments," but with increased emphasis on metabolic polymorphisms and their interaction with risk factors that determine individual susceptibility to adverse drug reactions, therapeutic drug efficacy, and cancer in relation to FDA's mission to regulate products and to protect public health.

Studies to identify genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy:

1. Metabolic polymorphisms, DNA repair, and individual cancer susceptibility.

Genetic and epigenetic regulation of cytochrome P450 1A2. Further studies are planned to elucidate the mechanism of gene regulation for cytochrome P450 1A2 (*CYP1A2*), given the importance of this enzyme as a determinant of therapeutic drug efficacy and colorectal cancer susceptibility. The Methylation-Specific PCR (MSP) method will be incorporated into this study which entails examining any group of CpG sites within a DNA region. This method allows the examination of the promoter region of the gene in normal and tumor tissue from the same individual using very small amounts of the human tissue. The mechanisms that control gene expression will be examined, including analysis of genetic variants and changes in gene methylation, an epigenetic mechanism.

Polymorphisms of cytochrome P450 1B1 and tissue-dependent expression. Studies on cytochrome P450 1B1 (*CYP1B1*), that is involved in both testosterone and estrogen hormone metabolism and in carcinogen and drug bioactivation, will continue with the use of well-characterized anti-peptide antibodies that the Division has developed and are using for immunohistochemical studies on tissue localization in humans. The identification of *CYP1B1* as the major P450 in human lung and the isolation of a large quantity of microsomes from a human lymphoma that selectively expressed high levels of *CYP1B1* will provide an abundant source of enzyme to conduct functional assays. In addition, discovery of a polymorphic variant of the *CYP1B1* gene that is associated with increased risk for prostate cancer will focus efforts on characterizing this variant enzyme for high or low activity or expression. These studies will be expanded to other hormonally related cancers such as breast and ovary, as well as lung cancer, and will provide fundamental knowledge on the therapeutic or potentially harmful effects of hormone therapies or oral contraceptive usage.

Polymorphisms of cytochromes P450 2A6 and P450 2E1. Other areas of research that are still in the early stages involve similar molecular biomarkers studies for esophageal and lung cancer, with special emphasis on the roles of cytochrome P450 2A6 (*CYP2A6*) and cytochrome P450 2E1 (*CYP2E1*), that are involved in the metabolism of carcinogenic nitrosamines that are often contaminants of the food supply and also present in tobacco smoke.

Polymorphism of phenol sulfotransferase. The major human phenol sulfotransferase (*SULT1A1*), which is present in human liver and appears to be a critical pathway leading to detoxification, exhibits polymorphic expression (see 1998 Accomplishments). Using a specific anti-peptide antibody and measurements of *SULT1A1* mRNA, we will examine this polymorphism and begin sequencing coding and/or 5'-regulatory regions of the *SULT1E1* gene. If this approach is successful, a genotyping method will be developed for use in assessing adverse health effects in colon, breast, and prostate cancer case-control studies.

Polymorphisms of glutathione S-transferases. Initial observations indicate a polymorphic distribution, at the protein level, of glutathione S-transferase (GST) A1 and GST A2, which are the major detoxifying enzymes in human liver catalyzing the inactivation of foodborne heterocyclic amines and mycotoxins. The Division has already measured the levels of these proteins in 30 human liver cytosols, and will soon begin sequencing the 5'-regulatory region of these genes, which are purported to be only 150-200 bases upstream of the coding region. If this approach is successful, a genotyping method can be developed that is expected to have wide applicability to individual differences in cancer susceptibility, adverse drug reactions, and chemotherapeutic efficacy.

The Division shall examine the inter-individual variation and details of tissue-specific GST expression. Quantitation of the GST phenotype of human pancreas has shown the expression of five forms of alpha class GST, *viz*; GST A1, A2, A4, A5 and A6, any one of which can be a major form in some tissues. All alpha class GST have glutathione-dependent peroxidase activity and A4 putative high activity towards hydroxynonenal and are thus, of considerable relevance to detoxification of lipid peroxidation products, high fat intake, and inadequate dietary antioxidants in relation to cancer risk. The Division entered into collaboration with Prof. Philip Board (Canberra, Australia) to facilitate investigation of these isoenzymes. Dr. Board has several GSTs, including A4 in recombinant expression systems. The enzymic properties and tissue distribution of GSTs of the theta and zeta classes will also be examined. In addition to continuing studies on GSTs of the mu class and of GST P1, as outlined in last year's report, we will investigate the theta and zeta classes. The theta class GST T1, is absent from approximately 30% of the population. It is associated with toxification reactions of organic halides. The possible role of *CYP450*, cyclooxygenases 1 and 2, and myeloperoxidase in polycyclic aromatic hydrocarbon metabolism in lung will also be examined.

GST P1 is the major GST in human bone marrow and can serve both to detoxify carcinogens and to conjugate chemotherapeutic agents. Thus, a collaborative case-control study is planned with investigators at the Arkansas Cancer Research Center (ACRC) to examine the frequency of the three GST P1 alleles in patients with a history of multiple myeloma, including those with recurrent disease and those who are disease-free after five years. This effort is based on preliminary observations that 75% of patients with active multiple myeloma possess the GST P1*B allele compared to only 35% of the general population. These studies are expected to yield the first insights into the etiology of this cancer and have the potential to improve the chemotherapeutic efficacy of drugs currently in use or in clinical trials, thus providing FDA with the mechanistic information needed for regulatory decisions.

The GSTs described above not only catalyze the conjugation of chemotherapeutic metabolites with glutathione, but also the cytotoxic products of oxidative damage created by radiotherapy. It is suspected that individuals with higher levels of GSTs may more quickly detoxify therapeutic and cytotoxic agents; thus, rendering certain cancer treatment paradigms less effective. In collaboration with pathologists and oncologists at the University of Arkansas for Medical Sciences (UAMS), we are evaluating the role of genetic polymorphisms in GST M1, GST P1, and GST T1 in response to therapy, as well as to levels of GSTs mu, pi, theta and alpha in tumor tissues. The ability to identify women who may be more or less sensitive to therapeutic agents could allow for adjustment of doses for treatment, or aid in other therapeutic decision making.

Inter-individual variation in DNA repair capacity. In 1999, the Division will also extend its examination of human polymorphisms to DNA repair enzymes by using the host reactivation base excision assay with cryoperserved human lymphocytes. Thus far, they have successfully prepared plasmid DNAs, adducted with aromatic and heterocyclic amines that are believed to play a role in urinary bladder and colorectal cancer and are in the process of establishing this assay in the laboratory. This approach will allow examination of inter-individual differences in DNA repair for carcinogen adducts that have been specifically implicated in the etiology of these human cancers, and can be extended to other agents suspected of being carcinogens in certain subpopulations. A portion of this work will be done in collaboration with investigators at the M.D. Anderson Cancer Research Center and the National Cancer Institute.

In addition, they will determine the impact of hormone replacement therapy (HRT) on DNA repair capacity, using a set of human lymphocytes collected in an ongoing study of gender and drug metabolism. Based on available literature, it is predicted that women on HRT will have a greater repair capacity than women not taking supplemental estrogen therapies. These studies will allow them to examine, in post-market studies, the potential enhancement of DNA repair in a subpopulation of women using FDA-regulated therapeutic estrogens.

Substrate specificity and activity of COX-1 and COX-2 toward metabolic activation of food-borne carcinogens. The Division shall continue to examine the human cyclooxygenases, COX-1, and COX-2, and their role in carcinogen bioactivation, especially in relation to colorectal cancer. The former is a constitutive enzyme necessary for gastric homeostasis, platelet aggregation and parturition and is expressed in many extrahepatic tissues. COX-2, on the other hand, is largely an inducible enzyme that is elevated in tissues by inflammation, cell proliferation, and cytokines. COX-2 also appears to play a critical role in colon cancer, with up-regulation associated with conversion of polyps to invasive adenocarcinomas. Both enzymes are subject to inhibition by non-steroidal anti-inflammatory drugs (NSAIDs), and the pharmaceutical industry continues to expend vast resources to market NSAIDs that may selectively inhibit COX-2. Specifically, the Division will examine the substrate specificity of COX-1 and COX-2 toward the metabolic activation of foodborne carcinogens and determine the levels of these enzymes immunochemically in colon tumors, colon polyps, and adjacent

normal mucosa. At the same time, they will characterize the carcinogen-DNA adducts present in these tissues and the efficacy of NSAIDs undergoing pre-market approval to inhibit these enzymes. They will pursue results from a series of preliminary studies that suggest that COX-2 expression may differ according to site of tumor origination, gender and tumor grade, indicating that chemopreventive strategies with COX-2 inhibitors may be less effective in certain subsets of individuals. These data are expected to aid FDA in the drug approval process and should provide additional insights into the etiology of metastatic colorectal cancer.

Gender-specific variation in drug metabolism. In 1999, the Division will also extend its examination of human polymorphisms to include the role of gender and hormonal status in inter-individual metabolic variability and the influence of exogenous hormonal therapies on drug metabolism. Thus far, they have initiated recruitment of eight subsets of 20 individuals each that represent both young and old men and women, and women who are taking oral contraceptives or hormone-replacement therapies. Specifically, they will examine the influence of the hormone/cytokine environment on the expression of the major *CYPs* in the liver with initial focus on *CYP1A2*. The information gained from this study will greatly aid in the interpretation of data from several ongoing projects aimed at understanding the mechanisms of individual-variability in drug metabolizing enzymes associated with hormonal variations in women. A better understanding of the molecular mechanisms responsible for individual-, gender-, or age-related variability in drug pharmacokinetics is a key determinant for postmarketing evaluations of therapeutic compounds and adverse drug reactions.

2. Chemoprevention.

Coffee and tea effects on *CYP1A2*, GSTs, and *N*-acetyltransferases (*NAT*s). Studies on chemoprevention will continue, based on recent data that tea components are potent inhibitors of *CYP1A2* and are expected to strongly affect drug disposition. In addition, data showing that the coffee lipids, kahweol and cafestol, not only induce GSTs but also appear to down-regulate *N*-acetyltransferases (*NAT*1 and *NAT*2), effectively changing the phenotype of rats from rapid to slow acetylators, will need further validation. In collaboration with the Division of Genetic Toxicology, the Division will use rat and human hepatocytes to explore the mechanism of *NAT* gene regulation. They are also planning pilot studies in humans to assess the effects of tea on drug metabolism and to examine similar effects of consuming coffee prepared with and without the use of filter paper, which quantitatively retains the coffee lipids. These results could have a profound impact on the ability to predict adverse drug reactions on the impact of tea and coffee consumption on drug bioavailability, and on colon and bladder cancer risk by foodborne carcinogens.

Tea extracts and components on *CYP1A2* , K-*ras, Mdr-1* and methyltransferase gene expression. An *in vitro* assay using human pancreatic and prostate tumor cells was developed to investigate chemoprotective mechanisms of black and green tea extracts and their components, which include the polyphenols, mixed theaflavins and the purified components, epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG). Studies on chemoprotective mechanisms will continue. Data have shown these

components to down-regulate K-*ras* and the multi-drug resistance gene, as well as to modulate methyl-transferase gene expression. These studies will continue with the use of a human liver hepatoma cell line, HU7 and hepatocytes transfected with the above gene, to further examine their chemoprotective properties.

DNA methylation, DNA methyltransferases, and homocysteine toxicity. The Division will continue to examine the role of physiological methyl donors and of DNA methylation in carcinogenesis, particularly in humans. Their recent improvements in *S*-adenosyl-methionine (SAM) and homocysteine analyses has permitted the use of non-invasive techniques to examine possible interactive effects between diet, genetic polymorphisms, and exogenous exposures on the DNA methylation status of individuals. An Interagency Agreement (IAG) with the National Cancer Institute (NCI) has been established to examine this question. Two genetic polymorphisms centered on abnormal methyl metabolism, catechol *O*-methyltransferase (COMT) and methylene-tetrahydrofolate reductase (MTHFR), have been found to be associated with altered cancer risk for breast and colorectal cancer, respectively. Their impact on DNA methylation status will continue to be examined. Collaborative studies are planned to compare methylation status in humans with susceptibility to cancer and other diseases. One involves the heat-labile form of MTHFR and its response to SAM inhibition, potentially resulting in concomitantly high homocysteine SAM levels in blood. Another collaborative study will examine possible defects in the regulation of the enzyme, DNA methyltransferase, and the development of human colon cancer. An alternate form of this enzyme activity has been observed in liver tumors of methyl-deficient rats. Like liver cancer in rats, the risk of colorectal cancer in humans is increased by dietary deficiency of methyl donors. Investigations into the abnormal gene methylation and expression contributing to carcinogenesis by methyl insufficiency will be continued, as will be the studies with Dr. M. Chou in the Division of Biochemical Toxicology on alterations in endogenous DNA adduct formation, DNA oxidation, and DNA hypomethylation in the livers of methyl-deficient rats. The genetic alterations responsible for cell transformation by methyl insufficiency have yet to be identified.

Methylation-specific PCR of the estrogen receptor and its relationship to colon cancer will continue to be investigated in 1999. Studies conducted this year have indicated that hypermethylation of the estrogen receptor in colon tumor tissue may influence the expression of cyclooxygenases, particularly the down-regulation of *COX-1*.

Epidemiology and post-market surveillance for chemical toxicants found in foods, drugs, cosmetics, and medical devices.

3. Etiology of human colorectal cancer: role of dietary heterocyclic amines.

In their ongoing study of dietary and genetic factors related to risk of colorectal cancer, the Division will continue to genotype or phenotype cases and controls for *CYP1A2*, *NAT2*, and *SULT1A1* activity. They will also apply the DNA repair assay using cryopreserved lymphocytes from the study participants, and plan to evaluate polymorphisms in other genes involved in the activation and detoxification of aromatic amines as they become available (*e.g.,* GST*A1*).

They are also collaborating with investigators at the Arizona Cancer Center to evaluate the role of heterocyclic amines and tobacco smoke carcinogens in polyp recurrence, and the possible modulating effects of *NAT1* and *NAT2* polymorphisms on risk associated with dietary consumption of heterocyclic and aromatic amines. The associations between exposures, susceptibility and *ras* gene mutations will also be assessed.

4. Etiology of human breast and prostate cancers in African-American and Caucasian men and women.

The Division has also begun case-control molecular epidemiologic studies of breast and prostate cancer in African-American women and men, as well as in Caucasians from the same locales. Because African-American men have the highest incidence of prostate cancer in the world, and African-American women have twice the risk of Caucasians for premenopausal breast cancer, the Public Health Service and the FDA Office of Women's Health are interested in evaluating possible genetic and environmental factors that may account for these racial disparities. These include dietary factors, particularly consumption of dietary heterocyclic amines, hormonal factors (oral contraceptives, hormone replacement therapy and reproductive hormones), and genetic variability in the metabolism of the heterocyclic amines and steroid hormones. These hypotheses will be applied to both breast and prostate cancer. Extensive questionnaire data, as well as blood specimens and urine for metabolic phenotyping, are being collected and will be evaluated for gene-environment interactions in relation to cancer etiology upon completion of data collection.

5. Etiology of human pancreatic cancer: role of carcinogen and drug exposures, chronic pancreatitis, and dietary imbalance.

The Division will continue to focus its attention on the analysis of molecular epidemiological data from their case-control study on pancreatic cancer which, like colorectal cancer, shares common risk factors that suggest the role of foodborne heterocyclic amines, including high meat and energy consumption, and low intake of cruciferous vegetables and fruits. Together with their data on pancreatic DNA adducts as described in "1998 Accomplishments," this project is expected to provide an assessment of the relative roles of dietary and environmental carcinogens in human pancreatic cancer and to result in appropriate recommendations for protecting public health.

In addition to the Division's continued focus on the analysis of molecular epidemiological data from this study, a major emphasis in 1999 will involve investigating the effects of nicotine and its metabolites, as well as other cigarette components, on normal and neoplastic human pancreatic cells. Cigarette smoking is the major risk factor of pancreatic cancer. Nicotine has already been shown to cause physiological and molecular alterations in rat exocrine pancreas. Thus, planned studies will determine the effects of nicotine and its metabolites on genetic (mutation) and epigenetic (methylation) events in exocrine and endocrine human pancreatic cells. The effects of nicotine on *CYP1A2*, on the protooncogenes H- and K-*ras*, the multi-drug resistance gene mdr-1, and on differential expression of other genes using RNA fingerprinting methods will be investigated. These studies will also examine the role of zinc in nicotine-induced effects. The activation and mutational profiles of the K-*ras* genes in normal, chronic pancreatitis, and neoplastic human pancreatic tissue grouped according to smoking status, gender and race will also be assessed.

Human exposure biomonitoring and DNA adduct detection.

6. Biomarkers of exposure and susceptibility for breast, prostate, ovarian, laryngeal, esophageal, lung, and urinary bladder cancers.

Another major emphasis in 1999 will be directed at the possible role of chemical carcinogens in breast cancer etiology, and the modification of risk associated with these exposures by polymorphisms in genes involved in carcinogen biotransformation. Because breast cancer most commonly arises from ductal epithelial cells, the Division began a study in 1998 to examine those cells shed into human breast milk for carcinogen-DNA adducts. They have been obtaining specimens from nursing mothers, both smoking and non-smoking, and have developed methodology to separate exfoliated ductal epithelial cells from human breast milk. In addition, they refined methods for DNA extraction from exfoliated ductal epithelial cells. In 1999, they are in the process of using ^{32}P-postlabelling/HPLC with synthetic standards to characterize DNA adducts present in human breast epithelial cells. Variability in adduct levels related to both exposure and to genetic susceptibility, based on variability in carcinogen metabolism, is expected. They are also collaborating with researchers at the Environmental Protection Agency who will evaluate mutagenicity of the same breast milk samples in an Ames *Salmonella* test that is sensitive to heterocyclic and aromatic amines. Researchers at the University of Guelph will be sent a portion of milk from the samples being evaluated to identify specific aromatic amines by GC/MS. The Division will study associations between exposures and susceptibility in relation to specific aromatic amines, mutagenicity, and DNA adducts in epithelial cells from the same samples. A study of chemical carcinogenesis in human breast epithelial cell studies, and of the effects of environmental and drug exposures and genetic susceptibility on these processes, are expected to have important implications for future FDA regulatory decisions. By sorting out etiologic mechanisms and putative risk factors in breast carcinogenesis, subgroups of individuals susceptible to specific carcinogens, particularly heterocyclic and aromatic amines, are likely to be identified.

A nested-case-series-study is also planned to identify and characterize DNA adducts in prostate tissue from men who are participating in the prostate cancer case-control study. As in the study of exfoliated ductal epithelial cells in human breast milk, the Division will evaluate levels of adducts in relation to environmental exposures as well as to polymorphisms in genes involved in metabolism of dietary and environmental carcinogens, as well as endogenous steroid hormones.

The Division is also beginning to focus on the role of oxidative stress, steroid hormone metabolism and exposure to environmental carcinogens and cancer risk in other hormonally responsive tissues. This includes a collaborative study with investigators at Memorial Sloan-Kettering Cancer Center. The Division will use epidemiologic data and biologic specimens collected in a recently completed study of ovarian and endometrial cancer to conduct this preliminary investigation of the role of metabolic polymorphisms in ovarian and endometrial cancers. The first specific aim of this study is to investigate associations between ovarian cancer risk and genetic polymorphisms in enzymes that may detoxify or contribute to the processes of oxidative damage to the cell, namely manganese superoxide dismutase (SOD2), glutathione S-transferase (GST M1), myeloperoxidase (MPO), and microsomal epoxide hydrolase (mEH). The second specific aim is to investigate associations between ovarian cancer risk and genetic polymorphisms in enzymes that metabolize classes of chemical carcinogens, including aromatic amines (*NAT1* and *NAT2*), and polycyclic aromatic hydrocarbons (GST M1, *CYP1A1*, and *CYP1B1*). For each of these enzymes, it is expected that differences in risk will be stronger in women with more ovulatory years. This analysis will be undertaken within the context of a case-control study of cancer that was conducted from 1994 to 1998. Cases were women with newly diagnosed cancer, aged 18 and over, identified at two hospitals in New York City, Memorial Sloan-Kettering Cancer Center, and New York Hospital. Blood samples from all cases and controls will be shipped to the Division of Molecular Epidemiology for extraction of DNA and genotyping. Case or control status will not be identifiable from the study numbers, so that the laboratory analyst will be blinded to case-control status.

In preliminary studies, the Division has obtained evidence that the heterocyclic amine, 2-amino-∀-carboline (A∀C), which is found in cooked foods and is the predominant aromatic amine carcinogen in cigarette smoke, is present as a major DNA adduct in human larnyx. During 1999, they plan to complete characterization of this DNA adduct and develop an LC/MS method, in collaboration with the Division of Biochemical Toxicology, to confirm adduct levels in human tissues. In addition, data indicate that acetaldehyde-DNA adducts may be formed *in vivo* after treatment with [^{3}H]ethanol. Recently, others have shown that acetaldehyde-modified DNA can be analyzed by ^{32}P-postlabelling (after reduction to ethylated adducts) and has been detected in human blood. Human epidemiological studies are also consistent with ethanol as a co-carcinogen, particularly when combined with tobacco usage. Of these, the relative risk for cancers of the upper aerodigestive tract, especially the larynx, show the most consistent synergism between total alcohol intake and heavy cigarette smoking. Thus, the Division proposes to examine the hypothesis that ethanol forms acetaldehyde-DNA adducts in human larynx and that these adducts may serve to enhance the relative persistence or mutagenic outcomes of A∀C and other smoking-related DNA adducts.

In collaborative studies in the area of molecular epidemiology, we are planning human studies are planned, with the UAMS and the German Cancer Research Center (Heidelberg) on the etiology of smoking-related lung cancer, with emphasis on the role of COX-1 and COX-2, as well as *CYP1A1*, *CYP2C9*, *NAT1*, *NAT2*, GST *P1*, and GST *M1*. In addition, they will focus on the relation between individual differences in these enzymes and the presence of endogenous carcinogen adducts and of those derived from smoking and from environmental exposures in human lung DNA.

A case-control study also is planned with the above-named centers to study the potential role of polycyclic aromatic hydrocarbons (PAHs) from diet, occupation, and tobacco smoke in the etiology of polyps and colorectal cancer. DNA adducts in colon polyp and tumor tissue will be evaluated in relation to questionnaire data, genetic and phenotypic polymorphisms in COX-1 and COX-2, *CYPs* 1A1, 1A2, 2C9, and 3A4 and glutathione S-transferases A1, A2, M1, M3 and P1.

Because of the Division's development of a rapid phenotyping assay for the phenol sulfotransferase (*SULT1A1*) in our colorectal case-control study (see 1998 Accomplishments), they will begin another collaborative case-control study with the University of Arkansas Medical School (UAMS) and the National Cancer Institute, funded through the inter-agency agreement, on urinary bladder cancer susceptibility. Like colorectal cancer, individuals with the low *SULT1A1* activity phenotypes are expected to be at elevated risk to aromatic amine bladder carcinogens found in cigarette smoke, foods, and the environment.

Extrapolation of the results of animal bioassays and of mechanistic studies to humans.

7. Evaluation of the neonatal mouse bioassay as an alternative bioassay for selected benzodiazepines, antihistamines, chloral hydrate, drugs inducing peroxisomal proliferation or oxidative stress, synthetic and natural estrogens, and endocrine disruptors, including chlorinated hydrocarbon pesticides and dinitroaniline herbicides.

In collaboration with the Division of Biochemical Toxicology at NCTR, ongoing studies involving the neonatal mouse bioassay (see "1998 Accomplishments") will provide data in 1999 on whether or not synthetic and natural estrogens and endocrine disruptors can serve as genotoxic carcinogens. In addition, they are planning to examine several recognized human carcinogens to validate more fully this model as a test for potential genotoxic human carcinogens.

International efforts in molecular epidemiology and biotechnology

8. Organization of the Molecular Epidemiology Group of the American Association for Cancer Research (AACR).

With the Division's role in establishment of the Molecular Epidemiology Group of the AACR (MEG-AACR), they will be involved in organizing scientific programs, symposia and conferences that will address public health concerns about individual susceptibility, especially in relation to the safety and efficacy of FDA-regulated products. In 1999, the Division will continue to play a major role in international harmonization efforts in molecular epidemiology and its application to human risk assessment.

9. Development and validation of DNA microarray technology for human diagnostics.

In its continuing studies on individual differences in drug and carcinogen-metabolizing enzymes, the Division has established a Cooperative Research and Development Agreement with Genometrix® to develop a "Risk-Tox" DNA microarray platform for rapid, high-throughput genotyping. The goal is to be able to genotype patients for all the major enzyme variants that would enable them to predict carcinogen susceptibility, adverse drug reactions, and perhaps chemotherapeutic drug efficacy. Such efforts could have a major impact on the ability to understand the likelihood of adverse health effects in susceptible subpopulations. In 1999, a major emphasis will be the completion of ongoing validation studies comparing our standard methodologies of genotyping to automated, large-scale genotyping on a robotic workstation and initiation of large-scale genotyping for ongoing epidemiologic studies. The immediate scientific importance of the research plan is two-fold. First, the development of a rapid screening methodology for genetic polymorphisms in risk-related alleles would greatly impact the rate at which data can be collected and analyzed in population-based risk-assessment studies**.** Currently, screening large sample sizes for a single allelelic variant by polymerase chain reaction restriction fragment length polymorphism (PCR/RFLP) analysis requires several weeks and numerous staff. Secondly, the development of rapid, automated screening methodologies (*e.g.*, analysis of several hundred alleles/500 persons/day) to identify individuals genetically at risk for adverse health effects would greatly facilitate FDA review of individual drug disposition studies and FDA post-market surveillance, based upon "profiles of individual risk" for agents with known toxicities in a given genetic background. The spin-off value for industry could have a revolutionizing effect on diagnostic medicine by allowing physicians to prescribe drug dosage more accurately and on an individual basis.

Significance to the FDA

These research projects are being carried out to identify human polymorphisms in carcinogen and drug metabolism, and to provide direct evidence for human exposure to specific chemical carcinogens. Furthermore, correlational analyses between DNA adduct levels and carcinogen-metabolizing enzymes in the same individuals allows not only the identification of populations who may be at higher risk for chemically induced cancers, but also provides evidence for the role of different chemical classes in human cancer etiology. Historically, FDA has based regulatory decisions on animal studies and on mechanistic data whenever available. However, these approaches do not take into account susceptible sub-populations, including children, genetically predisposed individuals, women, or specific ethnic groups. Future FDA regulatory actions will need to address sensitive subgroups and molecular epidemiology can provide a scientific basis for these decisions. Together, these efforts will surely result in better public health monitoring and regulatory risk assessment of FDA-regulated products and public health recommendations toward appropriate strategies for earlier disease diagnosis and cancer prevention.

NEUROTOXICOLOGY

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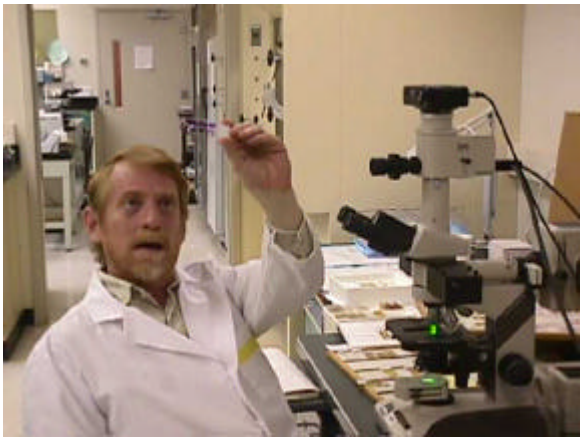

Dr. Larry Schmued, utilizes a fluorescent stain, Fluoro-jade, to identify dead or dying neurons.

Introduction

The congressional designation of the 1990's as the Decade of the Brain underscores the tremendous opportunities offered by the current and anticipated advances in brain research and the enormous cost of mental disorders to the national economy. In the United States, brain-related disorders account for more hospitalizations than any other major disease group, including cancer or cardiovascular diseases. One out of four Americans will suffer from a brain-related disorder at some point in their life-time, and the cost to the national economy for treatment, rehabilitation and related consequences is an estimated $400 billion each year. At no time in the past, however, have researchers been better poised to gain understanding of brain-related disorders and to reduce risks associated with neurotoxicity.

According to the congressional Office of Technology Assessment's April, 1990 report on neurotoxicity, “Neurotoxicity: Identifying and Controlling Poisons of the Nervous System,” the known or suspected causes of brain-related disorders include exposures to chemicals such as therapeutic drugs, food additives, foods, cosmetic ingredients, pesticides and naturally occurring substances. The number of potential neurotoxicants that will require FDA regulation has been estimated to be in the thousands and yet guidelines for neurotoxicity risk assessment remain vague and underdeveloped compared to those for cancer. Chemicals such as those listed above are also vital to the national economy and our daily lives are markedly improved by them. The problem is to determine at what dose and under what conditions a specific chemical may produce nervous system-related toxicity.

FY98 Accomplishments

The interdisciplinary approach, the use of multiple, established animal models and innovative biomarkers, and an in-depth working knowledge of and experience with mechanistically based focal areas of research enable the neurotoxicology research group to be responsive to FDA regulatory needs. There are several ongoing or planned studies, many in conjunction with other FDA centers, that exemplify the application of the group’s approach to providing critical research information applicable to FDA's

regulatory problems. The seafood neurotoxicant, domoic acid, and the prototypical excitotoxicant, kainic acid, are being evaluated as part of the excitatory amino acid focal research area in conjunction with colleagues at CFSAN and CDER. Progress to date includes the development and validation of neurochemical, neuropathological and behavioral methods for assessing alterations in amino acid neurotransmitters, dopamine release and specific neurohistological and behavioral indices associated with the N-methyl-D-asparte (NMDA)/glutamate receptor system. A cooperative research and development agreement (CRADA) was successfully negotiated with Astra Charnwood Pharmaceuticals to leverage FDA/NCTR resources. The objectives of the CRADA are to determine the effect of long- term NMDA receptor blockade on neurobehavioral endpoints in the developing monkey; to extend the research database with NMDA receptor blockers into the area of potential neuroprotective agents; and to utilize NCTR's nonhuman primate behavioral testing capabilities. Several publications describe the dose-response and age relationship of the seafood contaminant, domoic acid, exposure and the resultant lesions in the hippocampus, and other brain areas in the monkey, and neurohistological and behavioral alterations in the rat. These studies have been extended to examine the potential for developmental effects and to allow for the application of quantitative risk assessment procedures.

Considerable progress has been made towards the goals of developing, validating, and applying novel neurohistochemical markers. Fluoro-Jade, the novel histochemical tracer introduced in 1997 for the sensitive and reliable detection of neuronal degeneration, was further characterized. Specifically, it was shown to be a marker of amyloid plaques as well as degenerating neurons. The time course for the appearance of Fluoro-Jade-positive neurons following insult by excitotoxins, kainic acid and domoic acid, was studied. Fluoro-Jade was also used to detect and localize neuronal degeneration resulting from exposure to both aromatic monoamine releasers, methamphetamine and d-fenfluramine. A new homolog of Fluoro-Jade was also developed, Fluoro-Jade B. Preliminary studies indicate that it can result in an even higher resolution and more sensitive labeling of degenerating neurons than Fluoro-Jade. A different class of neurohistochemical tracer was also developed called Black-Gold. It was demonstrated that this novel aurochlorophosphate complex could be used as a simple and highly sensitive marker of both normal and pathological myelin. Myelin pathologies were documented following exposure to a number of FDA-relevant compounds including domoic acid, 3-NPA, and isoniazid.

Methods for assessing the neurotoxicity of the anorectic agent, d-fenfluramine, have been developed during the comprehensive study of amphetamine, methylenedioxymethamphetamine (MDMA) and methamphetamine (METH) under the monoamine focal area of research. These and other positive controls have been used to develop and validate the use of neurochemical monoamine concentrations, monoamine and excitatory amino acid release and receptor characterization, neuropathological (nerve terminal degeneration), and behavioral (spontaneous and operant) procedures for the quantitative assessment of the monoamine neurotransmitter systems. Furthermore, these data, and data on the influence of environmental temperatures and pharmacodynamics on neurotoxicity, have enabled a description of a more-defined mechanistic

pathway through which the neurotoxicity of substituted amphetamines produce neurotoxicity. Recently, rodent studies have demonstrated that core body temperature is a major determinant of the influence of d-fenfluramine on the serotonergic system in the brain. Data generated from multiple species exposed to a variety of doses of MDMA have been used to develop a biologically based, dose-response model for the quantitative risk assessment of neurotoxicants. This model, which allows the use of continuous data, is one of a handful of examples used by recent review committees (*e.g.*, the National Research Council and the International Life Sciences Institute (ILSI)) to exemplify quantitation of the risk assessment process for neurotoxicants.

The multispecies neurotoxicological assessments of several anti-HIV agents (*e.g.*, dideoxycytidine [ddC] and dideoxyinosine [ddI]) and the anti-tuberculosis agent, isoniazid, in conjunction with colleagues at CDER and the National Institute of Environmental Health Sciences (NIEHS), are nearing completion under the axonal transport/energy disruption focal research area. Neurophysiological (nerve conduction studies), behavioral (operant and spontaneous) and histological (glial fibrillary acidic protein [GFAP], immunocytochemistry, degeneration-specific stains and c-*fos* activation) methods have been developed to assess the effects of energy disruptors/transport inhibitors. Recently accepted and/or submitted manuscripts describe the first animal model of ddI-induced peripheral neuropathy and the associated time course of its histological effects.

In the oxidative stress focal area, data have been obtained that further support oxidative stress involvement in 3-nitropropionic-acid-induced neurotoxicity. A manuscript describing increased antioxidant enzyme activities following exposure to 3-NPA has been recently accepted for publication.

In cooperation with colleagues at CFSAN, the essential trace metal manganese is being evaluated with techniques developed for trimethyltin and methylmercury under the oxidative stress focal research area. The relationship between organometal-induced neurotoxicity (*e.g.,* methylmercury [MMT], triethyllead, trimethyltin) and oxidative stress has been examined with the newly developed *in vitro/in vivo* probe dichlorofluorescein. Generation of free radicals during oxidative stress has been correlated with lipid peroxidation, superoxide dismutase (SOD) transgenic alteration, changes in neurotransmitter receptor binding and alterations in cellular activity at the molecular level (c-*fos*, heat shock proteins). These techniques were also applied to other selective neurotoxicants such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine and will be utilized along with neurohistological methods and behavioral assessments of memory and learning. Recent publications describe evidence that inhibition of neuronal nitric oxide synthase by 7-nitroindazole-blocked, METH-induced dopaminergic neurotoxicity and neuronal nitric oxide synthase knockout mice are resistant to METH-induced neurotoxicity. Reports have also been published on the oxidative-stress-producing potential of manganese and the importance of valence on neurotoxic potency of metals.

The importance of developing appropriate animal models for use in interspecies comparisons of the effects of neuroactive agents has led to the development of automated systems for administering several complex behavioral tasks to laboratory animals as well as humans. These tasks are usually identical or very similar for all species. The maintenance of task continuity across species allows for the quantitative determination of similarities and differences in complex brain function and assists in the extrapolation of data from laboratory animals to humans. Additionally, the recent demonstration that performance of several of these tasks correlates significantly with IQ in humans, and selectively identifies attention deficit/hyperactive disorder (ADHD) in children serves to validate their use in studying important aspects of brain function in animals. Efforts have been increased at the NCTR's Complex Brain Function Laboratory at the Arkansas Children's Hospital to further define normative and clinical (i.e., ADHD) data for children performing NCTR's Operant Test Battery. In addition to the development and validation of the above-mentioned biomarkers of effect in the normal adult animal, the modulation of neurotoxicological outcome by age (development and senescence), nutritional status and body temperature have been frequently examined. Previous research has found that developmental cerebellar stunting results in hyperactivity, which is particularly prominent in males. The compounds investigated thus far result in a 5-10% decrease in cerebellar weight and include: retinoic acid, dexamethasone, and methylazoxymethanol. These effects are being evaluated as a possible animal model of ADHD. Future research includes investigating exposure to these compounds further with regard to progression of hyperactivity across maturity, assessment of social behavior, impulsivity, and attention.

The neurotoxicology research staff have enhanced scientific exchange by serving on several interagency committees as FDA/NCTR representatives. These committees include the Interagency Committee on Neurotoxicology, the FDA Intercenter Neuro-biology/Neurotoxicology Working Group, ILSI Working Group on Human Variability, and "Red Book II" FY98 revision. In addition, members of the staff have co-organized several national or international conferences such as the annual meeting of the Behavioral Toxicology Society and the "Third International Conference on Neuroprotective Agents" and "Cellular and Molecular Mechanisms of Drugs of Abuse" which resulted in published, peer-reviewed proceedings. These conferences have brought together scientists from government, industry and academia for information exchange and consensus building concerning methods development and risk assessment procedures for neurotoxicants.

FY99 Goals

The overall goals of neurotoxicology are to develop and validate quantitative biomarkers of neurotoxicity and to utilize them to elucidate toxic mechanisms. This will increase the certainty of assumptions underlying risk assessment for neurotoxicants. The strategy for achieving these goals has been to develop a multidisciplinary approach that integrates neurochemical, neuropathological, neurophysiological, and behavioral assessments to determine effects and mechanisms of neurotoxicity. The unique

features of the neurotoxicology research efforts at NCTR include the capability to determine target tissue concentrations and cellular interactions of neurotoxicants, and to reduce the uncertainty of extrapolating data across species by effectively using rodent and nonhuman primate animal models as well as humans whenever possible.

Over the last decade, expertise, equipment and facilities have been woven together to pursue the overall goals of neurotoxicology research through six primary objectives or focal research areas. These focal areas have been developed based on prevailing scientific understanding and on the importance of each area to regulatory concerns and include: 1) excitatory amino acids as mediators of aging and neuroanatomical susceptibility to neurotoxicants; 2) the role of aromatic monoamines in neurotoxicity; 3) disruptors of energy metabolism and axonal transport; 4) oxidative-stress-induced neurotoxicity; 5) interspecies extrapolation and validation of animal models, and 6) development, validation and application of novel neurohistochemical tracers. These focal areas include mechanistically based approaches for defining and understanding the potential for a broad range of drugs and other chemicals to produce neurotoxic effects. In some instances, the interaction of chemicals and age (development or senescence) have been investigated and this knowledge has been used to better understand developmental neurotoxicity and as an approach to elucidate the pathogenesis of neurotoxicants.

FY99 Plans

Several research projects in the various focal research areas are scheduled for initiation in FY99. Within the excitatory amino acid area, domoic acid-induced effects will be evaluated in the developing rat (collaboration with CFSAN). Some of the future goals concerning the development, characterization, validation and application of novel neurohistochemical tracers involve completion of 1998 work previously described. Specifically, completion of the chemical and biological characterization of Fluoro-Jade B and finalization of the studies showing Black-Gold and Fluoro-Jade labeling as a function of time following exposure to kainic acid. Another goal involves the development of a tracer which can be used to infer functional anatomy by simultaneously stimulating a brain region, while labeling the affected neurons and their afferent inputs. In the aromatic monoamine focal area, the influence of body temperature on d-fenfluramine as well as norfenfluramine-induced neurotoxicity will be further explored and studies on this and other stimulants (e.g., methylphenidate and ephedrine) will be completed (collaboration with CDER). In collaboration with CDER and pharmaceutical sponsors, the development of a monkey model of d-fenfluramine-induced cardiotoxicity (valvular changes) will be pursued. In addition, studies on the seizure-genic effects and neurotoxicity of amphetamine and other substituted amphetamines has been initiated through a newly approved protocol.

For the energy disruption focal area, data demonstrating the utility of animal models for the study of anti-HIV therapeutics (*e.g.*, ddI and ddC) will be published (collaboration with NIEHS and CDER). The time to onset of the histologically verified peripheral

neuropathy induced by ddI will be published. Two manuscripts that examine the fetal disposition of 3'-azido-3'-deoxythymidine (AZT) and dideoxy-didehydrothymidine (d4T), and another that evaluates the monkey as a model to study the peripheral neuropathy-producing effects of thalidomide and ddC, will be published in collaboration with NIEHS and CDER. In the area of developmental interactions with neurotoxicants, the Division will study neurotoxic effects of developmental and multigenerational exposure to estrogenic compounds in collaboration with NIEHS/NTP. Preliminary data suggesting that AZT is incorporated into fetal tissue after maternal administration will be augmented and submitted for publication.

In the oxidative stress focal area, studies of the effects of manganese on the nervous system in the adult and developing rat will be completed and published (collaboration with CFSAN). A recently approved protocol that focuses on the neurotoxicity potential of Ibogaine will be continued. In collaboration with CFSAN, 3-nitropropionic acid (3-NPA), a food-borne agent known to produce mitochondrial dysfunction, will be used in an attempt to develop a chemically induced rat model of ischemic-hypoxia. In order to validate the rat model of ischemic-hypoxia, further studies will be undertaken on the 3-NPA-induced neurotoxicity and the neuroprotective role of L-carnitine. In the interspecies extrapolation and validation of animal models focal area, validation studies on the acute effects of representative drugs in the NCTR Operant Test Battery will continue in the monkey and rat as will studies on the chronic effects of the prototypic drugs *(e.g.*, methylphenidate) used in the treatment of ADHD.

Development of neurotoxicological knowledge bases is an integral component of the overall scheme to derive predictive values for human risk. Knowledge bases are accumulations of data that have predictive values that reliably extend beyond individual data elements within a database. Predictive capabilities are achieved through the application of artificial intelligence programs such as neural networks, machine learning, expert systems, or other approaches currently being used and developed. The foundation of knowledge bases consists of biological endpoints (*e.g.*, neuropathological, neurophysiological, neurochemical, molecular biological and behavioral), data concerning mechanisms of action, structure-activity relationships (SAR), target-tissue concentrations, and physical/chemical properties of the agent. Hence, the prediction of human risk can be derived from the working model by assembling information in an ascending order of complexity from method-, agent-, or concept-driven research to strategies for prediction (*e.g.*, SAR and species extrapolation models) to databases. A complete database can be envisioned as the product of interactive and iterative processes between the several foundation components (*e.g.*, endpoints and mechanisms). In the process of developing knowledge bases from various data sources via quantitative risk assessment procedures, deficits in existing data will be identified that will determine directions for new research priorities. Subsequent studies can then be conducted to fill these identified data gaps to help complete the knowledge base.

Significance to the FDA

The importance of the interdisciplinary, mechanistically based approach of neurotoxicology research is that it encourages the development of in-depth, integrated knowledge bases and techniques that will be useful in addressing problems associated with current (e.g., thalidomide, fumonisin [FB1], domoic acid, methylphenidate, fenfluramine, ibogaine, and ephedrine) and future agents of regulatory concern.

As stated in the April 1990, Office of Technology Assessment (OTA) document on neurotoxicity, NCTR has the facilities, equipment and personnel to expand interdisciplinary research in neurotoxicity and to conduct research related to therapeutic drugs and food additives. Although neurotoxicology research at NCTR currently represents a major portion of FDA's neurotoxicology efforts, it must maintain its flexibility in order to deal effectively with future FDA needs. The following four-fold plan has been developed to allow neurotoxicology research to keep pace with FDA's responsibility to assure safe and effective drugs, foods, devices, and cosmetics. First, the Division must continue and enhance interactions with other FDA centers in order to better understand and address FDA regulatory concerns (*e.g.*, the FDA Intercenter Neurotoxicity Working Group). Second, they need to expand efforts in interdisciplinary and fundamental research approaches, especially in the molecular and interspecies areas, in order to validate appropriate animal models and quantitative risk assessment techniques for neurotoxicants. Third, they need to continue to develop and validate improved quantitative risk assessment procedures with broad applicability, and fourth, they need to continue to develop predictive system and knowledge base approaches to solve neurotoxicological problems. Integration of neurotoxicology research, FDA-wide, will provide the scientific basis necessary for sound regulatory decisions.

RESEARCH PROJECTS

RESEARCH PROJECTS

This section contains a listing of the NCTR research projects. Following is an example of each header as shown in the book, along with an explanation.

Project Number is a unique identifying number assigned to the NCTR projects. If the project has been changed, the identifying addendum number is located directly below the base number. The “E” number indicates a research project; the “P” number inidicates a preliminary experiment; the “S” number indicates a research support project; and the “X” number indicates a proposed project.

Principal/Co-Principal Investigator. The Principal Investigator (PI) is identified in bold type for each project. For a complete listing of principal investigators, see Index, "Projects by Principal Investigator."

Status/Research Area/Goal. Status: Indicates if the project was "Active" or "In Review" at the end of fiscal year 98, "Completed" during FY98, or "Proposed" for FY99.

Research Area (Res. Area): Abbreviates the NCTR research area responsible for conducting the project. The Division of Genetic and Reproductive Toxicology categorized their research into two laboratories, Genetic Toxicology (Gen Lab) and Reproductive Toxicology (Repro Lab). The Research Divisions are listed in the Table of Contents, with a complete listing of research, support, and contract areas located on page 203.

Goal: The abbreviation of the related NCTR strategic goals (listed in all capitals) are: PRED, prediction of toxicity; KNLG, knowledge based systems (computer-based); METH, method-driven research; AGNT, agent-driven research; CNPT, concept-driven research; and CTR SUP R, Center supported research. A more complete description of strategic goals is found in the Preface on page i.

Title. The acronym for the collaborating FDA Center, if applicable, is in parenthesis following the "Title." Below is a listing of the full names of the centers/offices.

Objective. A brief description of the purpose of the project. To locate a specific chemical related to a project, refer to the Index, "Chemical Index."

The FDA Centers/Offices:

Center for Biological Evaluation and Research (CBER)
Center for Devices and Radiological Health (CDRH)
Center for Drug Evaluation and Research (CDER)
Center for Food Safety and Applied Nutrition (CFSAN)
Center for Veterinary Medicine (CVM)
Office of Regulatory Affairs (ORA)

RESEARCH PROJECTS

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0023500	**Slikker**	Active/ **Neuro Tox**/ CTR SUP R	Primate Colony Surveillance	Determine the health status of the primate colonies maintained at NCTR.
E0026200	**Campbell**	Active/ **Micro**/ METH	Microbiological Diagnostic Methods: Development, Testing, and Evaluation	Improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology and serology as applicable to NCTR programs and projects.
E0213001 E0213011	**Delclos** Weis	Active/ **Bio Tox/** AGNT	A Comparison of Weight Gain and Fertility in CD Rats Fed a Standard Diet (NIH-31) or a Soy- and Alfalfa-free, Casein-containing Diet (NIH-31C)	Evaluate effects of NIH-31C on fertility by comparing pregnancy rates and litter size and weight in CD rats treated according to the treatment regimen to be used in the F0 generation of the multigeneration studies.
E0260401 E0260412	**Chou** Fu Chung	Active/ **Bio Tox/** CNPT	Effect of Caloric Restriction on DNA Binding and DNA Adduct Removal *In vivo*	Determine whether or not caloric restriction (CR) does 1. Affect the quantity of the total DNA adducts in livers from the mice treated with various carcinogens, namely aflatoxin B_1, (AFB1) benzo[a]pyrene (BaP) and 4-amino-azobenzene (4-AAB) or its methylated derivatives, and skin cells from the mice treated with BaP; 2. Alter the formation of the specific DNA adducts which may be responsible for the tumorigenicity of the chemical carcinogens; 3. Modify the efficiency of removal of the specific DNA adducts, either enzymatically or non-enzymaticallly; 4. Change activities of mouse-liver xenobiotic metabolizing enzymes, especially the hepatic glutathione S-transferase (GST) from the mice treated with AFB1 by measuring the *in vitro* and *In vivo* formation of AFB1-glutathione (GSH) conjugates.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0657300 E0657301 E0657302 E0657303 E0657304 E0657305 E0657306 E0657307 E0657308 E0657312 E0657318	**Fu** Herreno-Saenz Kadlubar Von Tungeln	Completed/ **Bio Tox/** PRED	Tumorigenicity of Nitro-Polycyclic Aromatic Hydrocarbons (Nitro-PAHS) and their Metabolites in the Neonatal B6C3F1 Mouse	1. Determine the tumorigenicity in the neonatal B6C3F1 mouse of a series of parent nitro-PAHs, and their ring-oxidized and nitroreduced metabolites, which have been found to be mutagenic in the *Salmonella typhimurium* strains. 2. Determine structure-activity relationships of nitro-PAHs, as well as to determine the structural features which can affect tumorigenicity. 3. Determine if bacterial mutagenicity correlates with tumorigenicity of the nitro-PAHs selected for study. 4. Compare and assess the importance of ring-oxidation pathways and nitroreduction pathways for the metabolic activation of nitro-PAHs in relation to tumorigenicity.
E0657500 E0657501	**Lewis**	Completed/ **AniH/DietP** METH	Nutrient Digestibility and Nitrogen Balance Among Fischer 344 Rats and B6C3F1 Mice Fed NIH-31 Standard and Fortified Diets	Determine the digestibility and utilization of various dietary nutrients in the NIH 31 study and fortified diets by F344 rats and B6C3F1 mice.
E0662700	**Shaddock** Arlotto Casicano Schol	Active/ **Gen Lab/** METH	Reliable Methodology for Cryopreservation	1. Develop a reliable methodology for cryopreservation of isolated hepatocytes. 2. Assess the effects of cryopreservation on hepatocyte cultures with studies designed to measure changes in morphology, viability, recovery, metabolism and ability to repair DNA after chemical treatment.
E0666900	**Fu** Von Tungeln	Completed/ **Bio Tox/** CNPT	Comparative Regioselective and Stereoselective Metabolism of 7-Chlorobenz[a]anthracene and 7-bromobenz[a]-anthracene by Mouse and Liver Microsomes	Study the effects of chloro and bromo substituents on the regio- and stereo-selective metabolism of benz[a]anthracene by mouse and rat liver.
E0667700	**Poirier** Lyn-Cook, B. Zapisek	Completed/ **Mol Epi/** CNPT	A Study to Determine if the Carcinogenic Effect of a "Methyl-Deficient Diet on Rats can be Reversed by a "Methyl-Sufficient "Diet"	Study methylation patterns of liver DNA and liver carcinogenesis in rats on methyl deficient diet then switched to methyl sufficient diet.

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E0671700	**Pipkin** Hinson Lyn-Cook, L.	Active/ **Gen Lab/** CNPT	The Stress Mobility Group of Proteins as Potential Biomarkers of Caloric Restriction In Aging Rats	Determine differences in High Mobility Group (HMG) proteins in young rats (*ad* lib); aged rats (*ad lib*), and aged rats (CR).
E0671900 E0671911 E0671921 E0671931	**Ali** Bowyer Carrington Lipe Melethil Newport Scallet Siitonen Slikker Sobotka Soliman Thompson	Completed/ **Neuro Tox/** AGNT	Neurotoxicity Assessment of Prenatal, Postnatal and Adult Exposure to Manganese (Mn) in the Rat (CFSAN)	1. Determine whether administration of Mn during prenatal, postnatal and adult periods produces: a. any significant accumulation of Mn in plasma and different regions of the rat brain, b. alterations in the dopaminergic neurotransmitter system, as evidence by (1) changes in concentration of dopamine and its metabolites; (2) in dopamine receptor binding and dopamine release, and (3) in the rate limiting enzyme tyrosine hydroxylase. 2. Determine if accumulation of Mn in the divalent (Mn+2) or trivalent (Mn+3) state is associated with producing neurotoxicity. 3. Determine if Mn accumulation and neurotoxicity is enhanced if administered to iron deficient rats during prenatal, postnatal or adult periods.
E0672700	**Scallet** David Nikonorov	Completed/ **Neuro Tox/** CNPT	Age Potentiation of TMT Neurohistological Toxicity in Rats	1. Confirm those portions of previous research which demonstrated a potentiating effect of age (7 vs. 17 months) on TMT-induced hippocampal neurohistological toxicity. 2. Extend findings by adding an older age group (24 months) and an intermediate age group (12 months) to the analysis which we hypothesize will demonstrate an increasing neurohistological toxicity of TMT with age. 3. Extend findings by including an additional neurohistological biomarker (c-fos protein expression) of TMT toxicity expected to reflect an earlier stage of neuronal damage.

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E0676700 E0676711	**Aidoo** Casciano Lyn-Cook, L.	Active/ **Gen Lab/** PRED	Development of an Assay to Measure 6-Thioguanine-Resistant Rat T-Lymphocytes Treated with Mutagenic Agents *In Vitro*	Develop techniques for *in vitro* mutagenicity studies with rat lymphocytes.
E0677500	**Delclos** Blaydes Heflich Jacobson	Active/ **Bio Tox/** AGNT	DNA Damage in Mammary Tissue, Liver and Nucleated Blood Cells of F344 Rats with Polyester Polyurethane (Microthane Foam) Implants (CDRH)	Develop methods for the detection of low levels of DNA damage produced by metabolites of 2,4- and 2,6- toluenediamine (TDA).
E0678600	**Cerniglia** Wang	Completed/ **Micro/** PRED	Microbial Studies on Macronutrient Food Substitutes Phase I: Validation Studies (CFSAN)	Validate the semicontinuous culture system for further studies on effect of macronutrient food substituents on the microbial activity and ecology of human intestinal microflora.
E0678800 E0678801 E0678820	**Feuers** Berg Duffy	Completed/ **Gen Lab/** AGNT	Acute Toxicity of Ganciclovir: Circadian Response and Effect of Dietary Restriction (CFSAN)	Determine if a circadian effect on the acute toxicity of ganciclovir can be demonstrated. A further goal is to determine if caloric restriction has an impact on the chromotoxicology of ganciclovir.
E0681600	**Aidoo** Lyn-Cook, L. Wamer	Active/ **Gen Lab/** CNPT	Studies on Antioxidants: Evaluation of the Mutagenic Activity of N-Ethyl-N-nitrosourea (ENU) in the Rat (CFSAN)	1. Pretreat F344 rats with antioxidant vitamins: Beta-carotene, L-ascorbic acid and dl-α-tocopherol in the drinking water for one week and then expose the animals to 100 mg/kg ENU (a direct acting mutagen) or to simultaneously expose the animals to the antioxidants and 100 mg/kg ENU; 2. Determine the tissue concentrations of the vitamins from the liver and the spleen after exposure to use the spleen lymphocytes to measure the frequency of 6-thioguanine-resistant T-cells employing the rat lymphocyte clonal assay to evaluate the relationship between ENU-induced *hprt* locus mutations and antioxidants intake.

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E0682200 E0682211	**Paule** Ali Binienda Ferguson Gillam Johannessen Slikker Sobotka Taylor	Active/ **Neuro Tox/** AGNT	The Effects of Chronic 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) Admin-istration on Complex Brain Function, Neurochemistry and Neurohistology in the Rhesus Monkey (CFSAN)	Chronic administration of low doses of the dopaminergic neurotoxicant MPTP will lead to detectable alterations in 'cognitive' brain function in the absence of frank Parkinsonian symptoms.
E0682500	**Wolff** Dunkel Jackson Whittaker	Active/ **Bio Tox/** AGNT	Determination of Dose Levels to be Used in Chronic Car-cinogenicity Study of Iron Overload (CFSAN)	Determine the dose levels of carbonyl iron to be used in a subsequent chronic bioassay
E0682900	**Lipe** Ali Carrington Newport Slikker	Completed/ **Neuro Tox/** AGNT	Effect of Manganese on the Concentration of Amino Acids in Various Regions of the Rat Brain (CFSAN)	1. Determine if exposure to manganese alters amino ac-ids concentrations in selected regions of the adult rat brain. 2. Determine if exposure to manganese alters amino acid concentrations in selecting regions of the developing rat brain.
E0683400 E0683401 E0683402	**Feuers** Deluca York	Completed/ **Gen Lab/** CNPT	The Effect of Age and Food Restriction on Glutathione S-Transferase (GST) Isozymes in C57BL/6N Mice	Seek correlation between aging, caloric restriction, and 1(3)+-Butyl-4-hydroxyanisole (BHA) administration with alterations in GST isozyme composition.
E0683700	**Paule** Gillam Slikker	Active/ **Neuro Tox/** AGNT	Effects of Chronic Methyl-phenidate (Ritalin) Admini-stration on 'cognitive' Func-tions in the Rhesus Monkey	Determine whether chronic treatment with relevant doses of the therapeutic agent methylphenidate (Ritalin) will result in detectable changes in specific 'cognitive' abilities in a nonhuman primate model of complex brain function.
E0684000	**Colvert** Ferreira Holland	Completed/ **Micro/** METH	Detection of *Clostridium botulinum* using Enzyme Linked Immunosorbent As-says and Polymerase Chain Reaction Techniques (ORA)	Develop better *in vitro* meth-ods for the detection of *C. botulinum*.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0684800	**Littlefield** Hass Poirier	Active/ **Bio Tox/** AGNT	The Effect of Dietary Magnesium (Mg) on the Induction of Tumors, Transformation of Cells, and Leukemia Incidence	1. Identify and evaluate the appearance of tumors, cell transformation, disruption of cell cycles and increased incidences of leukemia that may be related to dietary Mg deficiency, and 2. evaluate possible modulations of tumor expression through possible interactions of Mg and a carcinogenic metal, such as nickel (Ni).
E0685000 E0685011	**Aidoo** Heflich Manjanatha	Active/ **Gen Lab/** PRED	Lymphocyte Mutation as a Biomarker for Mammary Tumors Induced by 7,12-Dimethylbenz[a]anthracene in Sprague Dawley Rats	Mutation at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus of lymphocytes from Sprague-Dawley rats treated with DMBA can be used as a biomarker for the induction of mammary tumors.
E0685300	**Yerokun** Heflich	Active/ **Gen Lab/** PRED	Construction of Transgenic Hamster Ovary Cells Expressing Arylsulfotransferases IV (AST IV) and Their Use in Studies of Molecular Mechanism of Arylamine- and Polycyclic Aromatic Hydrocarbon (PAH) -induced Carcinogenesis	1. Construct a mammalian expression vector containing the AST IV gene and to transvect Chinese hamster ovary cells with the recombinant vector; 2. Use these transgenic cells in the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) and adenosine phosphoribosyl transferase (*aprt*) mutation assays.
E0686701 E0686711 E0686721	**Hansen, D.** Dial Grafton	Completed/ **Repro Lab/** PRED	Investigations on the Mechanism of Valproic Acid (VPA)-Induced Embryotoxicity *In Vitro*	Determine if 5-formyltetrahydrofolate, 5-methyltetrahydrofolate or folic acid is able to decrease the incidence of VPA-induced neural tube defects in rat embryos *in vitro*; To determine if L- or D-serine, formate, or Met is able to decrease the incidence of VPA-induced neural tube defects in rat embryos *in vitro*; To determine if pretreatment of rats with Met is able to decrease the incidence of VPA-induced neural tube defects in rat embryos *in vitro*.

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E0687001	**Ahn** Kodell	Active/ **Biometry**/ METH	Nonparametric Estimation and Testing of the Tumor Incidence Rate in Survival/ Sacrifice Experiments	Develop a method to estimate the tumor incidence rate under the constraint that the tumor incidence rate is non-negative.
E0687101	**Freni** Ahn Hine Turturro	Active/ **Biometry/** AGNT	Caloric Intake and Human Health (The NHANES-1 Study)	Investigate whether caloric consumption is a predictor of human health in general, or of certain specific health effects.
E0687401	**Miller** Freeman Grahn Heinze	Active/ **Chem/** METH	Development of Devices/ Methods for Determination of Food/Seafood Quality	Assist FDA with problems incurred in testing seafood for decomposition by developing an expeditious assay for determining volatile and semi-volatile organic compounds in spoiled seafood.
E0687511 E0687521 E0687531 E0687541	**James** Poirier Wise	Active/ **Bio Tox/** CNPT	ADDEND: Antioxidant Induced Changes in Rat Liver DNA Methylation Status	Determine whether the antioxidant Butylated Hydroxytoluene (BHT) (ionol) influences DNA methylation in the liver of rats.
E0687801 E0687811	**Lyn-Cook, L.** Aidoo Casciano Wamer	Active/ **Gen Lab/** CNPT	Evaluation of the Effects of Dietary Antioxidants on Lymphocyte Function and Genotoxicity Induced in Young and Old Rats Exposed to DNA-Damaging Agents *In vivo* (CFSAN)	1. Determine the effects of the antioxidant vitamins on the genotoxicity induced by exposing mutagens/carcinogens to young and old rats. 2. Determine the effects of antioxidant vitamins on lymphocyte function in mutagen-exposed and non-exposed young and old rats.

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E0687901 E0687912 E0687913 E0687914 E0687915 E0687916 E0687917 E0687918	**Fu** Beland Casciano Contrera Doerge Heflich Kadlubar Poirier Teitel Von Tungeln	Active/ **Bio Tox/** PRED	The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells CDER)	1. Determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs. 2. Examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo[a]pyrene, 6-nitrochrysene, and aflatoxin B_1 3. Study the metabolism and DNA adduct formation of benzodiazepine and antihistamine drugs by mouse and human liver microsomes to determine which if any cytochrome P450 is responsible for metabolic activation in mice and humans. 4. Transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes will also be employed to study the mutations and DNA binding of the subject drugs.
E0688101 E0688111	**Hansen, D.** Dial Grafton	Completed/ **Repro Lab**/ CNPT	Investigations on Carbamazepine (CBZ) Embryotoxicity *In Vitro*	1. Determine if exposure of embryos *in vitro* to carbamazepine (CBZ) alters normal growth and development. 2. Determine if a stable epoxide metabolite of CBZ or metabolic activation of CBZ by microsomes alters normal growth and development of embryos. 3. Determine if any of three folate derivatives will ameliorate potential embryotoxicity due to exposure *in vitro* to carbamazepine (CBZ) or its principle metabolite.

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E0688201 E0688211	**Wolff** Ali Contrera	Active/ **Bio Tox/** AGNT	Tumor Promotion and Neurochemical Changes in Mice During Chronic Feeding of the Anti-depressant Fluoxetine (CDER)	1. Determine if chronic feeding of fluoxetine (Prozac) results in promotion of mouse mammary carcinomas. 2. Determine if chronic feeding of fluoxetine: a) produces changes in the concentrations of serotonin and its metabolite, 5-hydroxyindoleacetic acid, in different regions of the mouse brain; b) induces changes in serotonergic receptor and uptake sites in different regions of the mouse brain.
E0688501	**Branham** Andrews Burroughs Doerge Fishman Medlock Sheehan Streck	Completed/ **Repro Lab/** KNLG	Effects of Therapeutic Antiestrogens on Postnatal Uterine Development in the Rat (CDER)	Assess the developmental toxicity of the antiestrogens toremifene, droloxifene, and ICI 164,384 in the developing rat uterus as measured by uterine weight, luminal epithelium morphology and ultrastructure, and uterine gland genesis. Assess uterine estrogen receptor modulation by neonatal antiestrogen exposure.
E0688601	**Streck** Branham Sheehan	Active/ **Repro Lab/** KNLG	*In Situ* Expression of Estrogen Receptor (ER) Protein and mRNA in the Developing Reproductive Tract	Analyze estrogen effects on ER levels in the developing reproductive tract at the cellular and molecular genetic level.

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E0688701 E0068711	**Ali** Cadet Freyaldenhoven Newport Slikker	Active/ **Neuro Tox/** CNPT	Evaluation of Constitutive and Stress-Induced Levels of Expression of Heat-Shock Proteins in Cu/Zn-Super Oxide Dismutase-Transgenic Mice	1. Determine whether there are significant differences in constitutive HSP expression in Cu/Zn-Super Oxide Dismutase-transgenic mice versus non-transgenic littermate controls, C57BL/6N controls as well as CD1 controls. 2. Determine whether there are significant differences in the expression of inducible forms of HSPs after exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in super oxide dismutase (SOD)-transgenic mice versus non-transgenic littermate controls, C57BL/6N controls as well as CD1 controls. 3. Determine whether there are significant differences in the timeframe of the HSP response in SOD-transgenic mice versus non-transgenic littermate controls, C57BL/6N controls as well as CD1 controls. 4. Determine whether there exists differential expression of isoforms of HSP in SOD-transgenic mice versus non-transgenic mice littermate controls, C57BL/6N controls as well as CD1 controls. 5. Evaluate if induction of HSP correlates with the depletion of dopamine in SOD-transgenic mice versus non-transgenic controls, C57BL/6N controls as well as CD 1 controls.

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E0688801	**Chou** Aidoo Allaben Bowers Casciano Gaylor Giri Green Hinton James Kodell Morris Roth Sahu Sotomayer Warbritton	Active/ **Bio Tox/** AGNT	A Collaborative Research Proposal to Assess Cancer Risk Posed by Intermittent Exposure to Aflatoxin B1 in Rats CFSAN)	1. Test the hypothesis that a chemically induced tumor incidence is a function of the accumulated lifetime exposure, and is predictable from the average daily dose for various dosing regimens, such as continuous and intermittent dosing. 2. Study correlation between the chemically-induced tumor incidence and various biomarkers of the initiation and the promotion stage of carcinogenesis for continuous and intermittent dosing.
E0689001 E0689011	**Streck** Fishman Rajaratnam Webb	Active/ **Repro Lab/** CNPT	Effects of Maternal Diabetes and Insulin on Fetal Expression of Insulin-like Growth Factor and Insulin-like Growth Factor Binding Protein mRNAs	Determine whether experimentally inducting diabetes in pregnant rats by treatment with streptozotocin will alter fetal expression of insulin-like growth factor mRNAs and insulin-like growth binding protein mRNAs. To determine to what extent restoring normoglycemia in pregnant diabetic rats by treatment with insulin will restore the normal pattern of fetal expression of insulin-like growth factor mRNAs and insulin-like growth factor binding protein mRNAs.
E0689101 E0689111	**Sheehan** Burroughs Faber Hughes Whitten	Active/ **Repro Lab/** KNLG	Alterations in Reproductive Tract Morphology and Biochemistry in Rats Treated Neonatally with Phytoestrogens	1. Determine if phytoestrogens, when given neonatally, alter estrogen receptor and progesterone receptor concentrations in the uterus and brain at 6 and 10 months in the same manner as diethydstilbestrol (DES). 2. Determine if phytoestrogens, when given neonatally cause the same morphological alterations in the female reproductive tract at 6 and 10 months as DES. 3. Determine if phytoestrogens, when given neonatally, elicit the same induction of the c-*ras*, c-*myc* and c-*fos* oncogenes as DES.

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E0689401 E0689411 E0689421 E0689431	**Teitel** Huber Kadlubar Lin	Active/ **Mol Epi/** PRED	Chemoprotection of DNA Adducts of 2-Amino-1-methyl-6-phenylimidazo-[4,5-b]-pyridine in the Rat	Examine the effect of the glutathione S-transferase inducers, phenethylisothiocyanate, diallyl sulfide (DAS), 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz), garlic powder, cabbage powder, 2(3)-tertbutyl-4-hydroxyanisole (BHA), kahweol palmitate, cafestol palmitate, quercetin, tannic acid, a-angelicalactone, Green tea, and ethoxyquin on the metabolism and DNA adduct formation of the food-borne carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine, in the Fischer 344 rat.
E0689601	**Kodell** Ahn	Active/ **Biometry/** METH	Attribution of Tumor Lethality in the Absence of Cause-of-Death Information	1. Develop a nonparametric procedure for estimating distributions of time to onset of and time to death from occult tumors in the absence of cause-of-death information. 2. Develop a method for entering the number of fatal tumors in an experiment that lacks cause-of-death data, in order to modify the IARC cause-of-death test. 3. Develop a procedure for estimating the lag time between onset of and death from an occult tumor, when cause-of-death data are unavailable. Illustrate the new procedures using data from the PCR studies.
E0690101	**Pothuluri** Assaf Bloom Cerniglia Nawaz	Active/ **Micro/** METH	Microbial Degradation of Drugs and Feed Additives Used in Fish Farming (Aquaculture) (CVM)	Develop a standardized method to evaluate the biodegradation of drugs and feed additives used in fish farming (aquaculture). Determine the biodegradation rates and metabolic fate of the antibiotic erythromycin in aquaculture water and sediments.

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E0690201	**Kodell** Chen, J.J. Lin	Active/ **Biometry/** PRED	Bioassays of Shortened Duration for Drugs: Statistical Implications (CDER)	Conduct a Monte Carlo simulation study to evaluate the effect that terminating rodent bioassays at 18 months (or earlier) instead of 24 months would have on the statistical power to detect carcinogenic human drugs.

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E0690301 E0690311	**Bowyer** Clausing Davies Gough Holson Newport Sandberg Slikker Stewart	Completed/ **Neuro Tox/** CNPT	Factors Affecting the Neurotoxicity of Amphetamines and Related Compounds	1. Determine how age, mode of administration, and environmental temperature during drug exposure alter the neurotoxicity of fenfluramine and methylphenidate. 2. Measure the effects of age and environmental temperature on the pharmacokinetics of several of the amphetamines. 3. The effect of neurotoxic doses of methamphetamine (METH) on the blood-brain barrier will be assessed to determine whether a "leaky" or damaged blood-brain barrier results from such exposure, and whether aging potentiates the likelihood of such damage. 4. The role of glia in METH and dexfenfluramine neurotoxicity will be assessed by elucidating the time-course of METH-induced gliosis, and by assessing the role of glia-derived neurotrophic growth factor (GDNF) in such neurotoxicity. 5. Neuroprotective compounds, neurotoxins, or compounds which affect energy utilization will be introduced into the striatum via microdialysis, while closely controlling body temperature, to determine if these compounds alter hyperthermia-induced METH neurotoxicity. 6. Ascertain whether the dopamine and serotonin depletions caused by continuous none acute exposure to low levels of METH via osmotic mini-pump are also dependent upon environmental and body temperature.

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E0690401 E0690411	**Ferguson** Holson	Completed/ **Neuro Tox/** PRED	Development of Techniques for Producing and Measuring Attentional Deficit Hyperactivity in Rats	1. Further development and characterization of existing techniques to detect behavioral hyperactivity. 2. Development of new behavioral techniques for assessing activity and attention in the rat. 3. Use of the above techniques to assess the impact of neonatal lead or dexamethasone exposure on activity and attention.
E0690501	**Ferguson** Gough Hansen, D. Holson Laborde Paule	Completed/ **Neuro Tox/** AGNT	Neural and Functional Teratogenesis of Retinoids in the Rat	1. Determine age-specific retinoid dosage levels which produce no more than a 20% reduction in viability and do not increase the incidence of major morphological abnormalities. 2. Identify and characterize the age-specific functional and neurological alterations produced by the above doses. 3. Assess within-animal and within-litter correlation between functional and underlying neurological abnormalities induced by retinoids.
E0690601 E0690611	**Manjanatha** Aidoo Casciano Heflich Lyn-Cook, L. Mittelstaedt	Active/ **Gen Lab/** PRED	Quantitative and Molecular Analysis of 7,12-Dimethylbenz[a]anthracene induced mutations in the model Blue Rat: Comparison of Mutagenesis in the Transgene *lacI* with the Endogenous gene *hprt* and Cancer Genes H-*ras* and P53	1. Determine the mutant frequency and mutation spectrum of the l*acI* transgene of the Blue Rat following exposure to DMBA in surrogate and target tissues and compare these mutant frequencies and mutational spectra to those determined in Objectives 2 and 3. 2. Determine the mutant frequency and mutation spectrum of the endogenous *hprt* reporter gene in T-lymphocytes from the spleens of Fisher 344 and Blue Rats following exposure to DMBA 3. Induce mammary tumors in Fischer 344 rats and Blue Rats by exposure to DMBA and screen tumor DNA for mutations in the oncogene, H-*ras* and the tumor suppressor gene, p53.

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E0690801	**Zheng** Kodell	Active/ **Biometry/** METH	Properties of the Hazard and Survival Functions of the MVK Stochastic Carcinogenesis Model	Investigate mathematical properties of the Moolgavkar-Knudson-Venzon (MVK) stochastic carcinogenesis model to deepen understanding and enlarge applicability of the MVK model. Study the two most important quantities of this model: the hazard and the survival function.Study the joint distributional properties of the numbers of initiated and malignant cells; Develop parameter estimation procedures so that the model can be fitted to real data; Exploit possible generalizations and extensions of this model.
E0691001	**Gaylor** Chen, J.J.	Active/ **Biometry/** PRED	Upper Limit for the Sum of the Risks of the Components in a Mixture and an Optimum Strategy for Risk Reduction	Develop a simple upper bound estimate of multiplicative risk factors and develop a simple upper bound estimate of the sum of the risks of components in a mixture. Utilize these upper limits to develop an optimum strategy for the expenditure of funds to reduce uncertainty in risk estimates.
E0691201 E0691211 E0691221	**Wolff** Ali James Whittaker	Active/ **Bio Tox/** AGNT	Cellular and Molecular Responses to Chronic Iron Overload in Animal Models (CFSAN)	1. Determine the health effects of chronic iron overload in mice and rats. 2. Determine neurochemical changes after chronic iron overload in mice and rats. 3. Develop an animal model for identifying the cellular and molecular mechanisms underlying the hepatic and pancreatic effects of chronic iron overload which are characteristic of the human disease idiopathic hemochromatosis and possible neurochemical mechanisms which associate effects of iron with neurological disorders, e.g., Parkinson and Alzheimer diseases.

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E0691401 E0691411 E0691421 E0691431	**Paule** Fogle Popke	Active/ **Neuro Tox/** PRED	Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity	1. Determine the acute effects of a variety of prototypic psychotropic agents on rodent performance in an operant test battery (OTB) containing tasks designed to model several complex brain functions. 2. Determine the relative sensitivities of the behavioral endpoints monitored in the rodent OTB to pharmacological disruption. 3. Compare and contrast the acute effects of these psychotropic agents on rodent and primate OTB performance to determine the degree to which behavioral findings in rodents can be extrapolated to primates. 4. Validate the use of rodent operant performance as useful predictors of neurobehavioral toxicity. 5. Add to existing knowledge of the neurochemical and neurophysiolgoical basis of complex brain functions.
E0691501	**Hansen, D.** Pauken Sonneborn Terry	Active/ **Repro Lab/** PRED	Stress Protein Expression Following Treatment with Developmental Toxicants *In Vitro*	1. Determine if mRNAs for stress proteins are synthesized by treatment with various developmental toxicants (valproic acid, lithium, ethanol, retinoic acid, and heat) in a rodent whole embryo culture system; determine the kinetics of stress protein mRNA syntheses; 2. Determine if this mRNA is translated into newly synthesized stress proteins and determine location of stress proteins in treated embryos by immunohistochemical detection.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0691801	**Terry** Hansen, D.	Active/ **Repro Lab/** PRED	Immunohistochemical Localization of Folate in the Neural Tube at the Time of Closure	1. Determine if folic acid and/ or 5-methyltetrahydrofolate is present in the neural folds at the time of closure of the neural tube in untreated mouse and rat embryos; 2. Determine if the location or quantity of folate present in the neural folds at the time of closure is altered by treatment with valproic acid which produces neural tube defects; 3. Determine if the location or quantity of folate present in the neural folds at the time of closure is altered by supplementation of the diet with folic acid.
E0692001 E0692011	**Doerge** Chang Churchwell Holder Rao	Active/ **Bio Tox/** CNPT	Toxic Hazards from Antithyroid Chemicals	1. Determine inhibition mechanisms for environmental goitrogens using purified thyroid peroxidase and lactoperoxidase; 2. Determine the mechanism for covalent binding suicide substrates to purified peroxidases using electrospray-mass spectrometry to analyze intact adducted proteins and/ or proteolytic fragments; 3. Determine mechanism of goitrogen uptake into isolated thyroid cells in primary culture and subsequent inhibition of iodination/coupling reactions involved in thyroid hormone synthesis; 4. Determine the structure-activity relationship for uptake of goitrogens into the thyroid and inhibition of thyroid hormone synthesis rats.

Project Number	**Principal/** Co-Principal Investigator(s)	Status/ **Res. Area/** GOAL	Title	Objective
E0692201	**Sutherland** Cerniglia Eppley Freeman Wilkes	Active/ **Micro/** AGNT	Microbial Metabolism of Fumonisins (CFSAN)	The hypothesis of this project is that certain micro-organisms have the ability to metabolize toxic fumonisins to other compounds, which may correspond to unknown mammalian metabolites. The objective is to identify the major microbial metabolites of fumonisins.
E0692301 E0692311 E0692321 E0692331	**Binienda** Ali Flynn Kim Rountree Scallet Slikker Wang	Active/ **Neuro Tox/** AGNT	3-Nitropropionic Acid (3-NPA) Hypoxia in the Rat: Neurochemical and Neurohistological Studies (CFSAN)	1. Evaluate the effects of the developmental neurotoxin 3-NPA on N-methyl-D-asparate (NMDA), dopaminergic and serotonergic systems using neurochemical methods. 2. Evaluate the neurohistological effects of calcium-mediated vs. serum-mediated stimuli on the expression of stress proteins (c-*fos*). 3. Correlate 3-Nitropropionic Acid (3-NPA) toxicity with age.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0692401 E0692411 E0692421	**Duffy** Allaben Chanderbhan Feuers Hart Hass Hattan Leakey Lewis Lyn-Cook, L. Lu Pipkin Tang Taylor Turturro	Active/ **Repro Lab/** CNPT	Effect of Different Levels of Caloric Restriction (CR) on Physiological, Metabolic, Biochemical, Immunological, Molecular, and Body Composition Variables in Rats	1. Determine how various levels and durations of CR affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, etc; 2. Determine the relationship between body fat (BF), fat free mass (FFM), total body water (TBW), and total body electrical conductivity (TOBEC) as a function of strain, age, mass, and nutritional status in rats; 3. Validate and automate the use of a new noninvasive electromagnetic scanning device to measure BF, FFM, and TBW and to compare the results to a conventional chemical fat extraction technique; 4. Determine if CR alters the relative quantity and disposition of various types of lipids such as cholesterol, phospholipids, free fatty acid, etc. in various tissues, as well as in urine, feces, and blood serum; 5. Develop control data related to CR that can be used by CFSAN to evaluate the toxicity and efficacy of low calorie foods, food additives, and food substitutes; 6. Determine temporal and environmental factors that modulate the effects of CR; 7. Develop experimental methods for utilizing a low level of CR to increase the survival rate and to decrease variability in the chronic bioassay; to provide the concomitant control data for comparison; 8. Develop control data for a reference purified diet that has been formulated to conform to a long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0692501	**Howard** Cashman Doerge	Active/ **Bio Tox/** CNPT	DNA Adduct Formation by Nicotine Metabolites	1. Determine the structural identity of the nicotine delta 1',2'- and delta 1',5'-iminium ion DNA adducts, and modify existing 32P-postlabeling techniques to detect the adduct. 2. Quantify the presence of these adducts *in vitro* and *In vivo* in mice.
E0692601	**Bowyer** Slikker Tank	Active/ **Neuro Tox/** METH	Implementation of Molecular Biological Techniques for Assessing Changes in Neurogrowth/Neurotrophic Factors after Exposures to Neurotoxicants and Other Substances	Select and produce/obtain cDNA and RNA probes for detecting changes in messenger RNA (mRNA) levels for the various neurogrowth/ neurotrophic factors (NTFs) which are likely to be involved in either secondary mechanisms of neurotoxicity or repair after neurotoxicant insult. Detect changes in NTF mRNAs after insult to neurotoxicants and other substances, and determine if these are the same for very young and older animals.
E0692701 E0692711	**Dobrovolsky** Heflich	Active/ **Gen Lab/** METH	Development and Validation of Mouse Embryonic Stem Cell Cultures for use in Generating Transgenic Animal Models with Targeted Transgenes	Mouse ES (embryonic Stem) cell lines will be established; The ability of ES cell lines to contribute to the germ line of mice will be determined.
E0692901	**Streck** Hansen, D.	Active/ **Repro Lab/** PRED	Toxicant Effects on Neural Cell Adhesion Molecule and N-cadherin during Mouse Neural Tube Closure	Determine the optimum time of neural cell adhesion molecule (NCAM) and N-cadherin expression in the closing CD-1 mouse neural tube; Quantitate changes in neural fold NCAM and N-cadherin levels following embryonic exposure to valproic acid, lithium or heat *In vivo*.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0693001	**Scallet** Ali Cairns Hall Johannessen Paule Rountree Schmued Slikker Sobotka	Active/ **Neuro Tox/** AGNT	Estimating Quantitative Neurotoxicity Risk from Domoic Acid Exposure (CFSAN)	Correlate pharmacokinetic profiles of single and multiple doses of domoic acid with associated quantitative neurohistological and behavioral effects in non-human primates; 1) To identify genetic factors modulating domoic acid sensitivity in Wistar rats; 2) To identify neurochemical biomarkers of domoic acid exposure and damage.
E0693101	**Wilkes** Cairns Chen, J.G. Fry Heinze Kaysner Lay Miller Rafii Sutherland Turturro Voorhees	Active/ **Chem/** KNLG	First Phase Development of a Rapid Screening Method for Identification of Complex Mixtures by Pyrolysis-Mass Spectrometry with Computerized Pattern Recognition (ORA)	Evaluate feasibility of the application of pyrolysis mass spectrometry (PyMS) with computerized pattern recognition (PattRec) for the rapid identification of a sample a) which is a complex chemical mixture, b) which is member of a set of such mixtures, and c) for which there is a regulatory need to distinguish the individual members of the set. Typical examples of applications: a) the rapid identification of culturable pathogenic and non-pathogenic bacteria in food, b) the distinction of adulterated from pure foods or cosmetics, or of generic from brand name pharmaceutical products, or c) demonstrating the virginity of plastic materials used in food containers.
E0693201	**Rafii** Cerniglia	Completed/ **Micro/** METH	Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) for Analysis of the Azoreductase Gene of Anaerobic Bacteria Isolated from the Human Intestinal Tract	Study the effects of genetic variations on the metabolic activities of azoreductases from bacteria isolated from human intestinal tract.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0693301 E0693311 E0693321	**Casciano** Harris Heflich Manjanatha	Active/ **Gen Lab/** PRED	Tumor Prone P53-Deficient Transgenic Mice (TSG-p53TM): A Potential System to Augment the Sensitivity of Carcinogenicity Testing and for Studying the Mutational Basis of Tumors	The genome instability of p53-deficient mice will be determined by monitoring the frequency of spontaneous mutations in the *hprt* biomarker gene of T-lymphocytes from the spleen. The time for appearance of tumors in the p53 heterozygotes will be compared with that for the wild type mice; *ras* and p53 mutations will be examined in such tumors. The frequency of mutations that arise on exposure of these animals to the carcinogens benzo[a]pyrene and dimethylnitrosamine in a neonatal carcinogenicity protocol will be monitored at the *hprt* locus in T-lymphocytes. The spectrum of carcinogen-induced mutations in the *hprt* locus will be determined by polymerase chain reaction (PCR) and DNA sequencing; this information may indicate mutational mechanisms, serve as a fingerprint of environmental exposure, and permit risk assessment.
E0693501	**Parsons** Heflich	Active/ **Gen Lab/** METH	Development of Methods for the Biochemical Selection of Mutations	Establish biochemical selection methods to detect and quantify rare mutations in the DNA of mutagen-treated animals. The value of the *E. coli* mismatch binding protein (Muts), used with the polymerase chain reaction (PCR), as a biochemical selection for mutations in the *ras* oncogene will be evaluated.

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E0693601 E0693611	**Ang** Churchwell Doerge Freeman Hansen, E. Luo Thompson	Active/ **Chem**/ METH	Development of Analytical Methods for Determination of Amoxicillin and Lincomycin in Fish Tissues	Develop highly sensitive analytical methods utilizing reversed-phase HPLC or GC for determining trace levels of amoxicillin and lincomycin residues in fish tissues. Specifically, the goal is to develop analytical methods which can be applied to determine amoxicillin in catfish muscle tissue and salmon muscle and skin tissues at 10 ppb and to determine lincomycin in salmon muscle and skin tissues at 100 ppb as suggested by the FDA Center for Veterinary Medicine (CVM). Separate procedures/solvent systems for the extraction, cleanup and HPLC analysis of each antibiotic are expected to be necessary because of the structural differences between amoxicillin and lincomycin. However, analytical residues in both the catfish and salmon tissue substrates will be developed if feasible.
E0693801	**Evans** Hanna	Active/ **Chem/** METH	Quantitative Determination of Enantiomers Composition and Purity of Drugs by Nuclear Magnetic Resonance (NMR) Spectroscopy (ORA)	1. Develop NMR methods to monitor enantiomeric purity of a group of Beta-adrenergic antagonists (i.e., propranolol, sotalol, pindolol and timolol.) The hypothesis is that effective NMR methods can be developed to monitor the enantiomeric purity of these drugs. 2. Develop NMR methods to monitor degradation products of a coronary vasodilator (nifedipine). The hypothesis is that effective NMR methods can be developed to monitor the degradation products of this drug.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0694101	**Roberts** Benson Howard Newkirk Tolleson	Completed/ **Bio Tox/** AGNT	Preparation of Antibodies Against the C1-C10 and Tricarballylic C14-C20 Segments of Fumonisin B_1. Development for Quantitative and Molecular Biological Techniques	1. Prepare fumonisin B_1-protein conjugates for immunization, immunoassay development, and epitope mapping; 2. Raise polyclonal anti-fumonisin B_1 adduct antisera and characterize titer, affinity, and relevant cross reactivity; 3. Evaluate the usefulness of anti-fumonisin B_1 antisera to elucidate target organ toxicity and as a tool to isolate or localize macromolecules modified by or binding fumonisin B_1; 4. Prepare immunoaffinity matrices and evaluate immunoaffinity techniques to enrich/concentrate/purify fumonisin B_1 as an aid to the identification and quantification of fumonisin B_1 in biologic samples including food.
E0694201	**Zhang** Ali Cerniglia Evans Freeman	Completed/ **Micro/** PRED	Microbial Transformations of Antidepressants	Establish a microbial system with a broad range of biotransformations as a model for mammalian drug metabolism of psychoActive/ compounds.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0694301 E0694311 E0694321	**Paule** Ali Binienda Clausing Gillam Slikker	Active/ **Neuro Tox/** AGNT	Behavioral and Neurochemical Effects of Short Course, High Dose Exposure to Methylenedioxymethamphetamine (MDMA) or dexfenfluramine (d-FEN) in Rhesus Monkeys	Establish acute dose-response curves for MDMA and d-FEN using performance of two groups of rhesus monkeys in the NCTR primate operant test battery (OTB)To produce long-term damage to the serotonin (5-HT) system of the forementioned monkeys via short course, high dose administration of MDMA or d-FEN. To determine whether rhesus monkeys exposed to short course, high dose MDMA or d-FEN exhibit persistent changes in CNS functioning, as quantified by changes in OTB performance. To determine if short course, high dose exposure to MDMA or d-FEN produces long-lasting changes in the acute effects of each drug (and hence long-term changes in CNS function) by establishing a second acute dose-response curve for each drug after such exposure. To demonstrate possible long-term changes in both neurochemical and behavioral endpoints resulting from MDMA and d-FEN exposure in rhesus monkeys that may assist in the determination of the status of these drugs as therapeutic agents.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0694501	**Doerge** Holder	Active/ **Bio Tox/** METH	Development of Methods for Analysis and Confirmation of ∃-Agonists	1. Develop determinative and confirmatory procedures using Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry (LC-APCI/MS) for multiresidue screening ∃-agonists in livestock tissues. 2. Develop synthetic procedures to produce authentic ∃-agonist standards for use in regulatory screening. These methods will provide the flexibility to adapt to the potential for "designer drug" modifications by clandestine laboratories. 3. Explore the use of packed column supercritical fluid chromatography (SFC) coupled to APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of ∃-agonists in livestock issues.
E0694601	**Kadlubar** Anderson Potter	Active/ **Mol Epi/** PRED	A Case-Control Study of Pancreatic Cancer and Aromatic Amines	Measure the associations of aromatic amine exposure and metabolism with the risk of pancreatic cancer. The sources of aromatic and heterocyclic amines to be studied are cigarette smoking and diet; the metabolic capabilities to be studied are acetylator status and N-oxidation status.
E0694701	**Kadlubar** Lang Lyn-Cook, B.	Active/ **Mol Epi/** PRED	Role of Acetylation and N-Oxidation in Colorectal Cancer	Confirm the initial findings of our pilot study regarding the roles of heterocyclic amine metabolism and exposure as putative risk factors from the diet or the environment. The sources of heterocyclic amines to be studied are cigarette smoking, diet and cooking methods; the metabolic pathways to be studied include heterocyclic amine N-oxidation status and O-acetylation status.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0694901 E0694911	**Pipkin** Hinson Lyn-Cook, L. Manajanatha Shaddock	Active/ **Gen Lab/** PRED	The Effect of P53 Null Phenotype on Bleomycin (BLM) induced Stress Protein Elicitation *In Vivo* in Transgenic Mice	1. Investigate the structure of the stress protein (sp) 70 and 90 genes by Southern blot in the 8-10 week old p53 null mouse in comparison with C57BL/6 control mouse; 2. Investigate the stress protein metabolic turnover (synthesis 35S-labeling) as a reflection of gene expression in the control homozygous C57BL/6 (+/+) and the null p53 homozygous TSG (-/-) mice as elicited by bleomycin (BL) at 1, 2, 3, 4 and 5 mo. of age (during the G1-phase of the cell cycle) by polyacrylamide gel electrophoresis (PAGE), and their levels of radio-labeling calculated by computerized electronic area measurements. If stress proteins (sps) are absent in bone marrow nuclei of 1 month old p53 null mice (sp synthesis is dependent on the presence of the p53 gene) or if their expression is below the level of measurement then the protocol will be discontinued at test group 1, see below. 3. Investigate the phosphorylation patterns of sps as a reflection of gene expression as elicited by BL using the same animal types, time frames and techniques as in Objective 1. 4. Identify and examine nuclear polypeptides other than sps for synthesis and phosphorylation levels as possible biomarkers of metabolic alterations and gene expression during phases of the cell cycle in control and homozygous p53 null mice following administration of BL.

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E0695001	**Manjanatha** Casciano Harris Shaddock	Active/ **Gen Lab/** PRED	Molecular Analysis of *In Vitro* Mutations in the Transgenic Rat2 Cells Exposed to Dimethylbenz(a)anthracene (DMBA) and Tamoxifen: Comparison of Mutagenesis in the Transgene *lacI* with the Endogenous Gene *hprt*	Determine the mutant frequency and mutation spectrum of the *lacI* transgene in Rat2 cells following exposure to DMBA and tamoxifen prior to evaluation in Blue Rat. To determine the mutant frequency and mutations spectrum of the endogenous hprt reporter gene in Rat2 cells following exposure to DMBA and tamoxifen. Compare *in vitro* mutant frequencies and mutational spectra with those determined in the Big Blue rats *in vivo* from E0690601.
E0695201	**Chou** Jackson James Poirier	Active/ **Bio Tox/** CNPT	Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin B_1 -Induced Carcinogenesis on Male F-344 Rats Fed Methyl-Deficient Diets	Study the interactions of dietary restriction (DR) and methyl deficiency (MD) on the alterations of hepatic oxidative DNA damages, DNA methylation, cell proliferation, oncogene and tumor suppressor gene mutation, preneoplastic foci formation and tumor incidence during the post-initiation stages of AFB1-induced carcinogenesis in male F344 rats. The results of these studies will: a) test the hypothesis that DR may be an antagonist to the promotional effect of MD in the AFB1-induced carcinogenesis; and b) evaluate the correlations between the effects of DR and MD on the formation of AFB1-induced preneoplastic foci and tumors and various biomarkers during the post-initiation stages of carcinogenesis.
E0695301	**Young** Bolon Branham Hass Meehan Sheehan Warbritton	Active/ **Biometry/** PRED	Rodent Embryo and Fetal Sectioning for Three-Dimensional Image Reconstruction and Animation	Develop the staining and sectioning techniques for conventional and laser scanning confocal microscopy to produce electronic images of rodent embryos and fetuses that can be used for computerized image morphing, 3D reconstruction, and animation.

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E0695401	**Freni**	Active/ **Biometry/** CNPT	Resting Metabolic Rate, Body Composition, and Dietary Assessment	1. Develop a prediction model for the resting metabolic rate (RMR) based on anthropometric data and body composition, and validated by measured RMR; 2. Collect dietary intake data and maximize their accuracy; identify potential sources of reporting of bias in relation to anthropometric data, and examine the correlation of calorie intake with RMR.
E0695501	**Griffin** Gollon Hobbs Kadlubar	Completed/ **Tech Adv/** AGNT	Accumulation of Manganese in Edible Muscle of Channel Catfish (*Ictalurus punctatus*) Following Exposure to Water Borne Potassium Permanganate	Determine the concentration and retention time of residual manganese in edible muscle of channel catfish after exposure to water borne potassium permanganate.
E0695601	**Jackson** Weis	Active/ **OD/Imm Off/** CNPT	An Evaluation of Dietary Fibers for the Prevention of Mammary Cancer in Female Rats	1. Develop an assay using 14C-estradiol to determine the amount of estrogen excreted via the feces by animals maintained on diets containing different types and levels of dietary fibers. 2. Use this assay, to evaluate several dietary fibers for their ability to increase estrogen excretion and to lower estrogen levels. 3. Test the most effective fibers in the dimethylbenz (a) - anthracene (DMBA)-mammary tumor model for their ability to inhibit tumor development at dietary levels shown to lower estrogen levels. 4. Establish if the inhibitory effect of dietary fiber on mammary tumor inhibition is dependent on the level of dietary fat.
E0695701 E0695711	**Young** Bolon Fleisher Hagstrom Laborde	Completed/ **Biometry/** AGNT	Changes in the Disposition of Methadone in Pregnant Rats and Their Fetuses	Conduct pharmacokinetic experiments in the non-pregnant, pregnant, and post-partum rat in order to quantify the differences in disposition of methadone.

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E0695801 E0695811	**Chen** Aidoo Casciano Heflich Manjanatha Mittelstaedt	Active/ **Gen Lab/** PRED	Mutant Frequencies and Types of Mutations Induced by Rat Carcinogens in the *hprt* and *lacI* Genes of Big Blue Fisher 344 Rats	1. Determine the mutant frequencies at the endogenous reporter gene *hprt* in T-lymphocytes from the spleens of Fischer 344 rats following exposure to five mutagens: aflatoxin B_1, N-hydroxy-2-acteylaminofluorene, benzo(a) pyrene, 2-amino-3-dimethylimidazo quinoline, and tris(1-aziridinyl)phosphine sulfide.; 2. Determine the mutant frequencies at the endogenous gene *hprt* and exogenous gene *lacI* from transgenic rats exposed to a mutagen selected from the five compounds examined in Objective 1; 3. Determine the types of mutations produced in the *hprt* and *lacI* genes in the mutants induced in Objective 2.
E0695901	**Rafii** Hehman Cerniglia	Active/ **Micro/** METH	Cloning and Characterization of the Genes Involved in the Metabolism of Nitro Compounds by *Mycobacterium sp*. Pyr-1	Understand the substrate specificity, cofactor requirement, and molecular characteristics of *Mycobacterium sp*. Pyr-1 nitroreductase and to determine the relationship of this enzyme to other microbial and mammalian nitroreductases involved in reduction of therapeutic nitro compounds.
E0696001	**Hansen, D.** Dial Grafton Terry	Active/ **Repro Lab/** PRED	Further Studies on the Mechanism of Valproic Acid (VPA) Induced Embryotoxicity	1. Determine a sensitive period for VPA induced neural tube defects (NTDs) in rat embryos treated *in vitro*; 2. Determine if VPA produces hypomethylation of DNA in treated rat embryos *in vitro*; 3. Determine S-adenosylmethionine/S-adenosylhomocysteine (SAM/SAHC) ratios in control and VPA-treated embryos during the sensitive period; 4. Determine if VPA produces hypomethylation of DNA in embryos treated with the drug *In vivo*; 5. Determine if inactivation of methionine synthase increases the embryotoxicity of VPA.

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E0696201	**Hammons** Blann Kadlubar Lyn-Cook, B.	Active/ **Mol Epi/** PRED	Methylation Profile, Gene Expression, and Enzyme Activity of CYP1A2 in Human Livers	Determine the possible involvement of epigenetic mechanisms in the regulation of the expression of the CYP1A2 gene. The methylation status determined for each sample will be correlated with the expression of the CYP1A2 gene and enzyme activity.
E0696301	**Delclos** Chen, J.J. Colvert Eaton Klimberg	Active/ **Bio Tox/** AGNT	Sexual Dimorphism in the Inflammatory Response to Biomaterials	Determine if a sex difference in the *in vitro* response of human monocytes and mouse peritoneal macrophages to various biomaterials can be demonstrated. Based on existing literature, we hypothesize that there will be a significant sex difference in the synthesis and release of inflammatory mediators that could influence the biocompatibility of the material.
E0696401 E0696411 E0696431	**Wolff** Cooney James	Active/ **Bio Tox/** CNPT	Prevention of Ubiquitous Synthesis of the Agouti Protein by Methyl Supplemented Diet	Test hypotheses that dietary methyl supplements fed to pregnant mice: 1. Increase expression of the pseudoagouti phenotype and decrease expression of the yellow phenotype among Avy/a offspring; 2. Have no adverse gross morphological effects on the offspring; 3. Increase the proportion of methylated cytosines in IAP promoter sequences in Avy/a offspring; and 4. Affect global DNA methylation levels and methyl metabolism in the dams and fetuses, as well as postnatal DNA metylation of the offspring.

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E0696501	**Erickson** Campbell Holland	Active/ **Micro/** METH	Development of an Improved Method for Determining the Tuberculocidal Activity of Chemical Disinfectants for Medical Devices	Develop an improved method for the rapid and accurate evaluation of the tuberculocidal activity of chemical disinfectants and sterilants. The hypothesis is that molecular methods can be used to (a) improve quantification of the disinfectant activity, (b) improve the reliability of the assay, and (c) shorten the time required for testing in comparison with the standard culture techniques. This protocol addresses the NCTR strategic research goal to conduct method-, agent-, or concept-driven research, through satisfying the need for an analytical method to accurately evaluate these products.
E0696701	**Littlefield** Chou Hass Mikhailova	Active/ **Bio Tox/** AGNT	Investigations into the Inter-Active/Oxidative Effects of Magnesium and Calcium with Selected Heavy Metals	Evaluate the influence of magnesium and calcium, both alone and in combination, on the toxicity from selected heavy metals in respect to the induction of oxidative DNA damage; Investigate the occurrence of adaptive responses in respect to the occurrence of oxidant stress from heavy metal toxicity; evaluate interactions of the anti-oxidant ascorbate in respect to oxidative damage from selected heavy metals; gain insight into mechanisms of action in regard to the toxicity and tumorigenic process instigated by heavy metals.
E0696901	**Baker** Sheehan Medlock	Active/ **Repro Lab/** KNLG	Enzymatic Oxidation of 17β-Estradiol Role of the Products in Hormone Action	Estradiol metabolites formed by peroxidase or tyrosinase interact with the estrogen receptor.

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E0697001 E0697011 E0697021	**Smith** Beland Fullerton Heflich Marques	Active/ **Bio Tox/** CNPT	Sequence Specificity of DNA Adduct Formation and Removal Following Chronic Carcinogen Administration	Determine whether or not certain nucleotide sequences bearing carcinogen adducts are more resistant than others to DNA adduct formation and repair, and to identify these sequences.
E0697101	**Ahn** Chen, J.J.	Active/ **Biometry/** CNPT	Tree-structured Over-dispersed Binomial and Over-dispersed Poisson Regression Models	1. Develop a tree-structured regression model to analyze over-dispersed binomial and over-dispersed Poisson data; 2. Develop an algorithm that extends tree-structured regression to the generalized linear regression model; 3. Identify the local effect of the covariates by stratified analysis of the data using tree-structured models; 4. Apply this method to developmental toxicity studies.
E0697401	**Arani** Chen, J.J.	Active/ **Biometry/** CNPT	Collinearity Under Proportional Hazards Model	1. Provide diagnostic tools to detect the presence of collinearity under proportional hazards and its quantitative effect on the results. 2. Provide algorithms to combat the harmful influence of collinearity, i.e., stabilize the parameter estimates and their variance. 3. Conduct a simulation study to examine the effectiveness of the algorithms.
E0697501 E0697511	**Aidoo** Bishop Heflich Lyn-Cook, L. Mittelstaedt	Active/ **Gen Lab/** PRED	Frequency and Types of Spontaneous Mutations Found in the *hprt* and *lacI* Genes of Lymphocytes from Transgenic Big Blue Rats	1. Determine the frequency of spontaneous mutation at the *hprt* and *lacI* loci in pre-weanling, young (4-month-old) and old (18-month-old) Big Blue rats; 2. Determine the types of mutations present in the mutants from Objective 1.

Project Number	**Principal/** Co-Principal Investigator(s)	Status/ **Res. Area/** GOAL	Title	Objective
E0697601 E0697611	**Laborde** Hansen, D. Hinson Lyn-Cook, L. Pipkin Shaddock	Active/ **Repro Lab/** CNPT	Dose-Response of Retinoic Acid (RA)-induced Stress Protein (SP) Synthesis and its Correlation with Developmental Toxicity in CD-1 Mice	1. Determine the incidence of limb malformations on gestation day 17 (GD-17) and the extent of synthesis of SPs in limb bud tissue determined 2.5 hr after RA treatment following various doses of RA administered on GD-11. 2. Determine incidence of cleft palate on GD-17 and the extent of synthesis of SPs in craniofacial tissue determined 2.5 hours after RA treatment following various doses of RA administered on GD-13.
E0697701 E0697711	**Chen** Burkhart Casciano Heflich Malling	Active/ **Gen Lab/** PRED	Evaluation of Chemical-Induced Mutagenesis in Transgenic Mice Containing the ϕX174 am3	Establishing the experimental parameters necessary to demonstrate a mutant frequency of 1.5- to 2-fold above background; Establishing the sensitivity of the am3 mouse model to mutagenic carcinogens and germ-cell mutagens expected to produce DNA damage at A:T base pairs. Where possible, compare the sensitivity of the ϕX174 system with that of other *in vivo* mutational systems; Establishing several basic properties of the ϕX174 am3 assay by determining the tissue or organ specificity of responses to certain carcinogens and by determining the patterns of mutations detected by the assay.

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E0697801 E0697811	**Ambrosone** Josephy Kadlubar Tang Thompson-Carino	Active/ **Mol Epi/** PRED	Chemical Carcinogenesis: Epithelial Cells in Breast Milk	1. Develop and refine a methodology for separation of luminal epithelial cells from human breast milk for DNA extraction; 2. Detect and quantify aromatic/hydrophobic-DNA adducts in luminal epithelial cells derived from human breast milk; 3. Detect genetic polymorphisms in carcinogen-metabolizing genes derived from DNA extracted from epithelial cells in human breast milk; 4. Evaluate the relationships between carcinogen-DNA adducts and smoking status, and adduct levels with polymorphisms in *NAT*1, *NAT*2, *CYP1A1*, and GST M1.

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E0697901 E0697901	**Paule** Gillam	Active/ **Neuro Tox/** PRED	Use of the NCTR Nonhuman Primate Operant Test Battery (OTB) as a Predictor of Acute Neurobehavioral Toxicity: Pharmacological Manipulation at Specific Neurotransmitter Receptor Subtypes	1. Further explore the extent to which the use of operant behavioral techniques in non-human primates can serve to reliably model the effects of compounds selected to act on specific neurotransmitter systems; 2. Determine the acute dose-effect relationships of several drugs believed to act primarily at subtypes of specific neurotransmitter receptors using rhesus monkey OTB performance; 3. Characterize the relative sensitivities of the various behavioral endpoints in the NCTR OTB to pharmacological manipulation of specific neurotransmitter systems and to add new tasks to the NCTR OTB; 4. More thoroughly characterize the role of specific neurotransmitter systems in the expression of complex brain functions through the pharmacological manipulation of specific receptor subtypes of some of the known major neurotransmitter systems; 5. Determine if the acute behavioral effects of the exogenous compounds of interest differ with regard to gender in the rhesus monkey.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0698001	**Hansen, E.** Ang Churchwell Doerge Luo Wilkes	Active/ **Chem**/ METH	Development of Methods for Analysis and Confirmation of Erythromycin A Residues in Tissue Samples from Terrestrial and Aquatic Farmed Animals by Liquid Chromatography	Develop determinative and confirmatory analytical chemical procedures, using high performance liquid chromatography/electrochemical detection and high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric detection, for Erythromycin A in biological samples taken from agricultural animals. Specifically, the goal is to develop complete methods for the analysis of Erythromycin A in muscle and liver tissues from poultry, non-processed bovine milk, and muscle tissues from salmon, catfish and shrimp. Sensitivity levels for these methods are expected to be at least 100 parts per billion for liver tissue and 50 parts per billion for muscle tissue and milk as requested by the Center for Veterinary Medicine.
E0698101	**Poirier**	Active/ **Mol Epi/** CNPT	Investigation of Short Term Dietary Methyl Supplementation in Manipulation of DNA Methylation and Methyl Metabolism in Mice	Determine whether short-term dietary methyl supplementation in mice will effect qualitative or quantitative changes in levels of methyl metabolites, levels of DNA methylation or levels of cell proliferation or apoptosis. Effects will be determined at two time points and at three levels of methyl supplementation. Provide data on some molecular and cellular events resulting from methyl supplemented diets. Provide specific new data and use new test strategies that will help us better extrapolate between human and animal data.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0698301 E0698311	**Ali** Duhart Hussain Klein Lipe Mukherjee Newport Rountree Sandberg Scallet Schmued Slikker Ye	Active/ **Neuro Tox/** AGNT	Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation with Neurohistological Evaluations in Mouse and Rat Brain (CDER)	1. Determine effects of ibogaine on dopamine, serotonin and their metabolite concentrations in different regions of mouse and rat brain. 2. Determine effects of ibogaine on reactive/oxygen species and lipid peroxidation in different regions of mouse and rat brain. 3. Determine effects of ibogaine on the activities of several antioxidant enzymes super oxide dismutase, catalase, glutathione peroxidase and glutathione levels in different regions of mouse and rat brain. 4. Evaluate effects of ibogaine on the activity of nitric oxide synthase in different regions of mouse and rat brain. 5. Determine levels of ibogaine, noribogaine and neurohormone, prolactin and corticosterone in plasma of mouse and rat. 6. Evaluate neurohistorical effects of ibogaine in different brain regions in the mouse and the rat and to correlate them with any neurochemical alterations.
E0698401	**Chen** Kodell Zheng	Active/ **Biometry/** METH	Statistical Analysis and Characterization of the Joint Actions of Toxicants	Develop a procedure for analyzing the quantal response data from a mixture experiment at a fixed total concentration; Develop a procedure for analyzing the survival data from a mixture experiment at a fixed total concentration; Develop a mixture model including both proportions and total concentrations; Apply the proportion-concentration model to characterize the joint actions of toxicants.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0698501 E0698511	**Hansen, D.** Dial Grafton	Active/ **Repro Lab/** PRED	The Role of Reactive/Intermediates in Carbamazepine (CBZ) -Induced Embryotoxicity	1. Determine if the antioxidant, glutathione (GSH), is able to decrease CBZ-induced embryotoxicity in mouse embryos; Determine if inhibition of GSH synthesis by L-buthionine-(S,R)-sulfoximine (BSO) increases the embryotoxicity of CBZ, determine if the antioxidative enzyme, super oxide dismutase (SOD), decreases CBZ-induced embryotoxicity; 2. Determine if the prostaglandin H synthase inhibitor, aspirin, decreases CBZ-induced embryotoxicty; 3. Determine if treatment with 12-o-tetradecanoylphorbol-13-acetate (TPA) which activates the release of arachidonic acid increases CBZ-induced embryotoxicity; 4. Determine if treatment with eicosatetraynoic acid (ETYA), an inhibitor of both prostaglandin H synthase and lipoxygenase decreases CBZ-induced embryotoxicity.
E0698701	**Roberts** Benson Doerge Gehring Newkirk	Active/ **Bio Tox/** METH	Tandem Immunochemical - Analytical Methods	Develop combined immunochemical and analytical chemical techniques to clean up complex matrices containing analytes of regulatory interest and provide detection at low concentrations with selectivity capable of providing structural confirmation.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0698801	**Wang** Cao Cerniglia	Completed/ **Micro/** METH	Development of a Universal Protocol for Detection and Identification of 13 Species of Foodborne Pathogens in Foods by Polymerase Chain Reaction (PCR)	Design PCR methods for detection and identification of 13 species of foodborne pathogens; Modification of the PCR methods to use same conditions including use of the same PCR cycler machine, same annealing temperature, and the same buffer system; Detection of the pathogens in various food samples by PCR; Development of a universal protocol for the PCR detection of the 13 species of foodborne pathogens in foods; Further improve the PCR specificity and sensitivity, and increase the species including other pathogenic *E. coli* and other non-anaerobic foodborne pathogens.
E0698901	**Billedeau** Churchwell Cooper Doerge Wilkes	Active/ **Chem**/ METH	Development of Methods for Analysis of Volatile and Non-volatile N-Nitrosamines in Relevant Cosmetics and Nitrite Cured Meat Products	Develop methods for extraction, cleanup, and analysis of non-volatile N-nitrosamines in cosmetics and meat products using combined liquid chromatography (LC) detection methods with confirmation by compatible mass spectrometry (MS) ionization methods; Investigate the applicability of Liquid Chromatography-electron spray ionization/mass sprectroscopy (LC-ESI/MS) and/or (LC-APCI/MS) as a multiresidue, trace level, quantitative technique for analysis of volatile, semi-volatile, and non-volatile N-nitrosamines in these consumer products.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0699001 E0699011	**Tang** Kadlubar	Active/ **Mol Epi/** PRED	The Role of Human Cytochrome CYP1B1 in Drug Metabolism and Carcinogenesis	Elucidate the role of human cytochrome P450 IB1 (CYP1B1) in drug metabolism and carcinogenesis. Specific aims are: Design and develop peptide-specific antibodies against human CYP1B1; 1. Determine levels of CYP1B1 protein in various human tissues; 2. Evaluate CYP1B1 expression as a biomarker for tumorigenesis; 3. Identify CYP1B1 inducers among the most common drugs and carcinogens; 4. Identify CYP1B1 substrates, including the endogenous steroid hormones, as well as drugs and carcinogens known to be metabolized by the closely related cytochromes P450 IA1 and IA2; 5. Find specific enzyme inhibitors for CYP1B1; 6. Develop a sensitive, convenient, and specific assay method for CYP1B1 enzyme activity *in vitro*; 7. Evaluate genetic polymorphism(s) for CYP1B1 as an epidemiological marker for cancer risk.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0699101	**Feuers** Aidoo Desai	Active/ **Gen Lab/** PRED	Influence of Dietary Restriction on Somatic Mutation and Antioxidant Enzymes Induced by Exposure of Female and Male Fischer 344 Rats to Bleomycin (BLM)	Determine the frequency of occurrence of lymphocytes bearing a mutant form of the *hprt* gene as an indicator of DNA damage in caloric restricted and in *ad libitum* rats following exposure to bleomycin (BLM); determine how the activity of antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione reductase relates to the mutant frequencies determined from the above objective; To determine the activity of the electron transport systems as an indicator of mitochondrial function during drug exposure; and evaluate the integrity of mitochondrial DNA in BLM treated rodents.
E0699201	**Patterson** Binienda Duhart Kim Lipe Slikker	Active/ **Neuro Tox/** PRED	Validation Study of the Physiologically-Based Pharmacokinetic (PBPK) Model for Description of low-dose, long-term Exposure of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Dosimetry in the Central Nervous System (CNS) (CFSAN)	Obtain CNS pharmacokinetic profiles of 2,4-D transport in the rat after low-dose, chronic dosing (28 days). The data will be used to validate the previously developed PBPK model which simulates the uptake, distribution, and clearance of 2,4-D.
E0699301 E0699311	**Delclos** Blaydes Chen, X. Sams	Active/ **Bio Tox/** KNLG	Evaluation of Host Factors Contributing to Differences in the Response to Biomaterials	Examine model systems that may be useful in the study of factors that regulate the extent of, and adverse effects arising from, the response to foreign bodies. As oxidative stress, including oxidative DNA damage, may play a major role in the foreign body reaction and in certain long-term adverse effects that may be associated with that reaction, we will also evaluate the utility of the air pouch model of inflammation to study species and strain differences in the development of and response to oxidative DNA damage.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0699501	**Thompson-Carino** Ambrosone Kadlubar McDaniel Tang	Active/ **Mol Epi/** PRED	Characterization of Ovarian-Specific Biotransformation of Estradiol A Model for the Identification of Inter-individual Variability in Tissue-Specific Steroid Metabolism	With current widespread use of hormone-based therapies and the increasing support for hormone-based chemoprevention therapies for breast cancer, concern regarding the role of estrogens, antiestrogens, and progesterones in the etiology of and/or progression towards cancers of hormonally-responsive tissues has continued to remain controversial in the cancer literature. Numerous studies, both epidemiological, as well as animal exposure studies, strongly suggest a role for estrogens in the carcinogenic cascade of several hormone-responsive cancers. It is predicted that the identification of genetic variability in estrogen metabolism among individuals can be utilized as biomarkers to assess cancer risk in large population-based epidemiological studies, providing a tool to address more directly concerns regarding the association of estrogens/-estrogen-metabolites, hormonal-based therapeutics and carcinogenesis in the human population.
E0699601	**Morris** Chen, J.J. Domon McGarrity	Active/ **Gen Lab/** CNPT	Evaluation of the Genotoxic Potential of Genistein in Human Lymphoblastoid Cells	Confirm the potential mutagenicity of genistein utilizing the *tk*/*hprt* mutation assay; Determine if apoptosis can account for the toxicity of genistein; Characterize the effect of genistein exposure on the traverse of the cell-cycle; Evaluate the role of the p53 tumor suppressor gene in the response to genistein exposure by performing the experiments which address objectives 1, 2, and 3 in both the AHH-1 *tk* (p53) and L3 (*tk*; p53) human lymphoblastoid cell lines.

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E0699701	**Miller** Freeman Heinze Holcomb Lansden Lay Thompson Wilkes	Active/ **Chem**/ METH	Innovative Methods for Determining Food Quality: Decomposition, Safety and/or Economic Fraud (ORA)	Examination of the total volatile bases (TVB) and putrescine (PU), cadaverine (CD) and histamine (HS) methods for potential regulatory use and validation of TVB as an indicator of decomposition; Develop rapid detection methods for the determination of decomposition analytes in seafood.
E0699801	**Hart** Feuers Leakey Lu Lyn-Cook, L. Pipkin Turturro	Active/ **OD/Imm Off** PRED	Memphis Study: Evaluation of Calorically Restricted Human Surgical Samples Received from Department of Surgery University of Tennessee, Memphis	Determine whether rodents and humans behave biologically in the same manner when calorically deprived but nutritionally supplemented.
E0699901	**Kim** Cerniglia	Active/ **Micro/** PRED	Biochemical and molecular analysis of polycyclic aromatic hydrocarbon (PAH) degradation by bacteria	1. Characterize multiple genes for the initial aromatic dioxygenase from F. yanoikuyae B1; 2. Determine putative common roles of ferredoxin and reductase components of initial dioxygenase in mono- and polycyclic aromatic hydrocarbon degradation; 3. Determine roles of the NahD (2-hydroxychromene-2-carboxylate isomerase) and NahE (cis-o-hydroxybenzylidenepyruvate aldolase) in polycyclic aromatic hydrocarbon degradation by *S. yanoikuyae* B1; 4. Determine molecular basis for polycyclic aromatic hydrocarbon degradation by *Mycobacterium sp*. PYR-1
E0700101	**Nawaz** Cerniglia Depaola Khan	Active/ **Micro/** CNPT	Purification and Characterization of Antibacterial Protein from Oysters (CFSAN)	1. Purification of the antibacterial protein from oyster homogenate; 2. Physical, biochemical, immunological and molecular characterization of the protein; 3. Determination of the kinetics of the inhibitory reaction.

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E0700201 E0700211	**Valentine** Burkhart Fane	Active/ **Gen Lab/** PRED	The Development of Transgenic Mice Harboring Bacteriophage ϕX174 with Sites Specific for Detecting Mutations at Guanosine: Cytosine Nucleotides, Small Frameshifts, and Extended Deletions	Find specific mutations in bacteriophage ϕX174 that render the bacteriophage non-infectious and that will revert to plaque-forming ability only when mutation occurs by specific mechanisms: 1. base substitution at a G:C base pair or; 2. frameshift caused by deletion of one or two nucleotides. An additional objective is to determine the feasibility of using ϕX174 to detect; 3. The deletion of an extended sequence. Phage harboring these mutations will be used to construct a transgenic mouse model for measuring mutations *In vivo*.
E0700301	**James** Hart Muskhelishvili Pogribny	Active/ **Bio Tox/** CNPT	Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy	1. In Nitroso methylurea (NMU)-initiated mammary epithelial cells, to determine whether nutritional manipulation of the cell cycle combined with low dose chemotherapy will permanently eliminate p53-independent and p53-dependent preneoplastic and neoplastic cells. 2. Determine the mechanisms of cell death induced by nutritional manipulation and low dose chemotherapy by examining molecular endpoints associated with p53-dependent and independent pathways of apoptosis.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0700401	**Fu** Von Tungeln Yi Yin	Active/ **Bio Tox/** PRED	A Study of the Secondary Mechanisms of Carcinogenesis: Lipid Peroxidiation and Endogenous DNA Adduct Formation from Chloral Hydrate, Benzodiazepines, Antihistamines, and Other Chemicals	Study secondary mechanisms of carcinogenesis, including lipid peroxidation and endogenous DNA adduct formation, for determination of the mechanisms by which chemicals, such as FDA regulated drugs including benzodiazepines and antihistamines, may induce cancer, and for the continued development of the neonatal mouse bioassay as a regulatory alternative tumorigenicity bioassay: 1. Develop analytical methodologies for analysis of lipid peroxidation products and endogenous DNA adducts; 2. Determine whether or not the drugs of FDA interest, including benzodiazepines and antihistamines studied in E687901, and other chemicals induce lipid peroxidation and endogenous DNA adduct formation *in vitro*; 3. Determine the inhibitory effect of lipid- and water-soluble antioxidants on drug-induced lipid peroxidation and endogenous DNA adduct formation *in vitro*; 4. Determine whether malondialdehyde-modified MG-1 DNA adduct and/or other endogenous DNA adducts can be used as biomarkers of lipid peroxidation; 5. Determine mutagenicity of the benzodiazepine and antihistamine drugs in *Salmonella typhimurium* TA 104 and determine whether mutagenicity in *Salmonella typhimurium* TA 104 can be used as a biomarker of lipid peroxidation induced by chemicals that generate free radicals upon metabolism.

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E0700501	**Lay** Darsey Heinze Holland Miller Rafii Sutherland Vorhees Wilkes	Active/ **Chem**/ METH	Rapid Identification of Intact Whole Bacteria Based on Spectral Patterns Using Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) (CFSAN)	1. Evaluate potential use of matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) as a method for the rapid identification of whole bacteria, either by comparison with archived reference spectra or by co-analysis with cultures of known bacteria; 2. Establish a standard set of conditions for the acquisition of MALDI-TOF mass spectra from bacteria suitable for use in bacterial identification. 3. Obtain some measure of the distribution of signals (ions at specific masses) obtained using standard MALDI-TOF MS conditions based on the analysis of a variety of related and unrelated bacteria. 4. Use standard (pattern recognition) as well as newer (artificial intelligence and principal components analysis) mass spectral recognition techniques to evaluate whether or not the standardized mass spectra obtained from bacteria are sufficiently distinct to allow identification of specific bacteria or to select related bacteria from a group. 5. Evaluate use of mass spectral recognition techniques for the identification of bacteria from mixtures based on MALDI-TOF MS analysis of the mixture. 6. Determine minimum number of bacteria necessary for obtaining standard mass spectra. 7. Evaluate effects on the reproducibility of spectra obtained from whole bacteria under different conditions of sample handling, storage, and cell growth.

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E0700601	**Gehring** Churchwell Cooper Doerge Holcomb Rushing Thompson-Carino	Active/ **Chem**/ METH	Development of Multiresidue Methods to Determine and Confirm Sulfonamides in Edible Tissues of Aquacultured Species	Develop analytical chemical methods to determine and confirm sulfonamide (SA) residues at the 1-10 ng/g level in edible tissues of aquacultured species. Technologies used will include liquid chromatography (LC) with post-column derivatization and fluorescence detection for the determinative procedure and liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (LC-APCI/MS) for the confirmatory procedure.
E0700701	**Rafii** Cerniglia Sutherland	Active/ **Micro/** CNPT	Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds	1. Detection of various metabolites of phytoestrogens, produced by the metabolism of these compounds by pure culture of bacteria typical of that isolated from human microflora, and elucidation of the metabolic pathways of phytoestrogens by human intestinal bacteria; 2. Assessment of the estrogenic effect of each phytoestrogen metabolite produced by intestinal bacteria; 3. Determination of the bacterial species producing estrogenic metabolites from phytoestrogens and elucidation of enzymes involved in various steps of these metabolic processes; 4. Effects of phytoestrogens and their metabolites on the population, composition, metabolic activity and enzyme production of bacteria from the human gastrointestinal tract.

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E0700811 X90046	**Sheehan** Lu Branham Tang	Active/ **Repro Lab/** AGNT	ADDEND: Bioassay of Reproductive Tract Toxicities Caused by Genistein and Methoxychlor in Sprague-Dawley Rats	It was determined that support for the feeding portion of E0700801 should include the use of the In-Life System for maintaining a record of body weights and feed consumption. The first group of rats to be entered into the In-Life System will be delivered to the study on 12/17/97.
E0700901	**Arani** Chen, J.J.	Active/ **Biometry/** METH	Analysis of Multiple Tumor Sites	1. Develop analytical and numerical techniques for computing the experiment-wise error rate in testing of multiple tumor sites; 2. Evaluate and compare the experiment wise error rate and power of various methods of p-value adjustment and recommend an optimal method for test of site-specific effects; 3. Evaluate the experiment wise error rate and power of global statistics for an overall test of carcinogenicity; 4. Recommend optimal procedures, which control the experiment wise error rate and still maintain the power, for the analysis of multiple tumor sites.
E0701001	**Binienda** Ali Kim Nickols Rountree Scallet Slikker	Active/ **Neuro Tox/** CNPT	Metabolic Correlates of the Neurotoxicity Associated with Exposure to the Mitochondrial Inhibitor 3-Nitropropionic Acid (3-NPA) in the Rat: The Role of Free Fatty Acids (FFA) (CFSAN)	1. Evaluate the acute effects of the mitochondrial inhibitor 3-NPA on brain metabolic activity using elecrophysiological, neurochemical, and neurohistological endpoints: a) spontaneous electrical brain activity and averaged visual evoked potentials; b) FFA concentration in different brain regions; c) brain regional monoamine neurotransmitter concentrations: dopamine, serotonin, and their metabolites; d) microscopically detectable neuronal damage; 2. Assess possible neuroprotective effect of L-carnitine in the rat model of 3-Nitropropionic Acid (3-NPA) induced histotoxic hypoxia.

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E0701111	**Beland** Gamboa Marques	Active/ **Bio Tox/** AGNT	ADDEND: DNA Adducts of Tamoxifen	As discussed in preliminary data, there appear to be two major DNA adducts formed in rat liver. One adduct appears to arise from a-hydroxy tamoxifen, while the identity of the other is presently unknown. This addendum proposes that a metabolite of a-hydroxy-N-desmethyltamoxifen is responsible for the second major adduct detected in rats treated with tamoxifen.
E0701201	**Tolleson** Wolff	Active/ **Bio Tox/** CNPT	Molecular Basis of Tumor Promotion and Increased Somatic Growth in Yellow Avy/a Mice: Mitogenic Effects of Agouti Protein *In Vitro*	Determine whether or not the agouti protein stimulates mitogenesis *in vitro*.
E0701301	**Schmued** Ali Bowyer Scallet Slikker Wang	Active/ **Neuro Tox/** PRED	Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants.	1. Develop and validate a battery of conventional and novel histochemical techniques for resolving the nature, distribution and underlying mechanisms of brain damage resulting from exposure to FDA relevant neurotoxicants; 2. Localize throughout the central nervous system, histochemical and pathological changes resulting from exposure to different classes of neurotoxicants, and 3. By correlating a compound's putative mode of action with a characteristic histochemical profile, develop the ability to predict the neuroanatomical regions at risk and the potential functional consequences of the neurotoxicant of interest.

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E0701401	**Aidoo** Desai Lyn-Cook, L. Manajantha McGarrity Morris	Active/ **Gen Lab/** PRED	The Use of Antioxidants in Single and in Mixture to Study the Effects of Dietary Vitamins on Genotoxicity Produced in Rats Treated with the Mammary Carcinogen 7-12-Dimethylbenz(a)anthracene and the Radiometic	1. Determine the genotoxic activity of dimethylbenz(a)anthracene (DMBA) and bleomycin (BLM) by the cytokinesis-block micronucleus and *hprt* assays in Fischer 344 rats that have been given a mixture of vitamin C, vitamin E, and B-carotene and selenium by gavage; 2. Determine the mechanism underlying the inhibitory action of the dietary antioxidants by determining their effects on: a) spectra of induced mutations in *hprt* gene in lymphocytes, b) oncogene (H-*ras*, K-*ras*) and tumor suppressor gene, p53 expression, c) programmed cell death (apoptosis), d) the activities of glutathione peroxidase, and glutathione S-transferase during DMBA and BM exposures.
E0701501 E0701511	**Ambrosone** Coles Stone	In Review/ **Mol Epi/** PRED	The Role of Glutathione S-Transferase genetic Polymorphisms in Breast Cancer Sensitivity to Radio- and Chemotherapy	1. Determine expression of enzymes (phenotype) in tumor tissue from women who received adjuvant therapy for breast cancer, using biopsy or surgical tissue specimens, using immunohistochemistry, and to evaluate associations between phenotypes in tumor tissue and risk of breast cancer recurrence.; 2. Determine inherited GST M1, GST T1 and GST P1 genotypes in normal tissue from these same women, and to determine associations of GST M1, GST T1 and GST P1 genotype with phenotype in tumor tissue; 3. Evaluate if GST genotypes predice breast cancer recurrence following treatment, controlling for other factors that may relate to prognosis.

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E0701701	**Lyn-Cook, B.** Blann Hammons Kadlubar	Active/ **Mol Epi/** PRED	The Effects of Low Zinc Levels on Ras, Mdr-1 Gene Activation and on Metabolic Enzyme Activities in Normal and Neoplastic Human Pancreatic Cells: A Possible Risk Factor for Pancreatic Cancer	Determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*. Examine ras, mdr-1, CYP1A1 and CYP1A2 expression in normal and neoplastic human pancreatic tissue grouped according to race and sex obtained from a human tissue bank.
E0701801	**Dobrovolsky** Heflich	Active/ **Gen Lab/** PRED	Validation of the Mouse Targeted tk+/- *In vivo* System for Use in Mutagenicity Studies	1. Expand a colony of transgenic *tk*+/- mice using breeding of *tk*+/- founders and C57Bl/6 mice, and to transfer the *tk*+/- genotype to a C57Bl/6 background; 2. Determine spontaneous mutant frequencies at the *tk* and *hprt* loci of splenic T-lymphocytes for mice of different ages; 3. Induce mutations in *tk*+/- transgenic mice using treatment with the point mutagen N-ethyl-N-nitrosourea (ENU) and the clastogens BLM and γ-radiation, and to measure the kinetics of mutant induction at the *tk* and *hprt* loci; 4. Breed transgenic *tk*+/- parents in an attempt to derive *tk*-/- knockout mice, and study the biological significance of the *tk* gene in mice; 5. Determine how the *tk*-/- genotype may effect mutant frequencies at the *hprt* locus.

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E0701901	**Binienda** Chatta Hardin	Active/ **Neuro Tox/** CNPT	Experimental Autoimmune Prostatitis: Implications for the prevention and treatment of Inflammatory and Neoplastic Disorders of the Prostate Gland	1. Induce an experimental autoimmune prostatitis in male rhesus monkeys by immunizing animals with homogenates of monkey prostate gland admixed with Freund's adjuvant; 2. Identify the target proteins of the induced autoimmune prostatitis by using the immune sera (IgG) from the above animals to: a) screen prostate homogenates by Western immunoblot analyses and b) screen a monkey prostate cDNA expression library.

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E0702001	**Hansen, D.** Grafton Streck	Active/ **Repro Lab/** PRED	Antisense Knockouts of Genes in the Folate Pathway and Effects on Neural Tube Development	1. Determine if knocking out 5,10-methyltetrahydrofolate (MTHFR) activity in mouse embryos *in vitro* produces neural tube defects; 2. Determine if addition of exogenous 5-methyltetrahydrofolate is able to overcome the lack of MTHFR activity and produce closed neural tubes in mouse embryos treated *in vitro*; 3. Determine if addition of exogenous methionine is able to overcome the lack of MTHFR activity and produce closed neural tubes in mouse embryos treated *in vitro*; 4. Determine if knocking out methionine synthase (MS) activity in mouse embryos *in vitro* produces neural tube defects; 5. Determine if addition of exogenous methionine is able to overcome the lack of MS activity and produce closed neural tubes in mouse embryos treated *in vitro*; 6. Determine if exogenous vitamin B12 is able to overcome the lack of MS activity and produced closed neural tubes in mouse embryos treated *in vitro*; 7. Determine if knocking out methionine adenosyltransferase (MAT) activity in mouse embryos *in vitro* produces neural tube defects; 8. Determine if addition of exogenous methionine is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated *in vitro*; 9. Determine if addition of exogenous 5-methyltetrahydrofolate is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated *in vitro*.

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E0702101	**Ambrosone** Green Hine Kadlubar Lang Stone Thompson-Carino	In Review/ **Mol Epi/** METH	Determinants of Indolent and Invasive Prostate Cancer	1. Determine levels of carcinogen exposure in African Americans and Caucasians with histologically confirmed prostate cancer using a case-control design; 2. Evaluate variability in hormone metabolism and susceptibility to carcinogen exposure, as measured by phenotypic and genotypic variability in carcinogen metabolism, and evaluate the interaction of these factors with the exposure data obtained in Specific Aim 1; and, 3. Characterize DNA adducts in prostate tissue from men with prostate cancer to identify mutagenic agents and evaluate levels of adducts in relation to carcinogen exposure data and susceptibility factors obtained in Specific Aims 1 and 2.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0702401	**Bowyer** Binienda Davies Ferguson Newport Peterson Schmued Slikker	Active/ **Neuro Tox/** CNPT	Evaluation of the Neurotoxic Effects and Determination of the Mechanisms of Induction of Limbic Seizures Produced by Amphetamine and Related Compounds	1. Measure the effects of dose and age on the susceptibility of amphetamine-induced limbic-type seizures in three different strains of rat and mouse, and identify areas in the brain, in particular the limbic system, where cell death and neuroplastic changes occur after amphetamine-induced seizures. 2. Determine the seizure genic capabilities of amphetamine, phentermine, methylphenidate and ephedrine in rat and mouse, the extracellular brain levels of these compounds necessary to induce seizures, and whether hyperthermia plays a role in the seizure induction. 3. Determine via brain microdialysis if extracellular glutamate levels are elevated in the limbic system (hippocampal rudiments and piriform cortex) prior to and during seizures induced by amphetamines. 4. Elucidate the role the noradrenergic, as well as the glutamatergic, system plays in seizures generated by amphetamines. Furthermore, begin to determine how agonists and antagonists of these two neurotransmitter systems can potentiate the seizure genesis of amphetamine.
E0702601	**Slikker** Binienda Bowyer Doerge Paule Schnellman	Active/ **Neuro Tox/** PRED	Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey (CDER)	1. Determine if the rhesus monkey demonstrates cardiac valve changes due to chronically administered dexfenfluramine; 2. Determine if the rhesus monkey demonstrates neurobiological changes due to chronically administered dexfenfluramine

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E0702701	**Dalu** Delclos	Active/ **Bio Tox/** AGNT	The Effects of Dietary Genistein on the Growth of Chemically-Induced Mammary Tumors in Ovariectomized and Intact Rats	This proposal will determine whether or not, in the absence of endogenous ovarian estrogens, dietary genistein can promote or suppress the growth of neoplastic mammary tissue at various stages for the carcinogenic process.
E0702901	**Delongchamp** Jacobson	Active/ **Biometry/** AGNT	Mortality Among Atomic Bomb Survivors who were Exposed *In Utero*	1. Estimate the dose-response relationship between non-cancer mortality and radiation exposure; 2. Assess the effect of gestational age at exposure on mortality; 3. Appraise the role of severe mental retardation in mortality.
E0703001	**Zheng** Kodell	Active/ **Biometry/** METH	Combining Carcinogenesis Models with Pharmacokinetic Models	1. Explore methods for using physiologically based pharmacokinetic models as tools for allowing target dose to be directly incorporated into stochastic carcinogenesis models, and hence improve risk assessment for various kinds of carcinogenic chemicals 2. Within the context of using combined models, investigate the feasibility of estimating certain biological parameters from data, if such parameter values are not readily available in the literature.
E0703101	**Chelonis** Paule	In Review/ **Neuro Tox/** AGNT	Decision Making in Children with Attention Deficit Disorder	Investigate the effects of methylphenidate on impulsivity in children using a delay of gratification procedure that has been used extensively in animal and human research. It is also the purpose to compare this delay of gratification procedure with other, more widely used, measures of impulsivity.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0703201	**Chelonis** Paule	In Review/ **Neuro Tox/** AGNT	Memory in Children with Attention Deficit Disorder	Investigate the effects of methylphenidate on short-term-memory using a delayed matching-sample procedure. This procedure has been used extensively to assess memory using both humans and nonhumans.
E0703301	**Chelonis** Paule	In Review/ **Neuro Tox/** AGNT	Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery	A battery of automated tests (games) will be given to measure aspects of learning, short-term-memory and attention, motivation, time perception, and color and position discrimination.
E0703501	**Hansen, D.** Chen, J.J. Fisher Fitzsimmons Gaylor Kimmel Laborde O'Conner Vega	Active/ **Repro Lab/** PRED	Predictability of Animal Data for Human Developmental Toxicity(CDER)	1. Retrieve reports of human data from published literature and FDA files for therapeutic agents for which there are adequate data to indicate either positive effects or no effect; 2. Retrieve reproductive and developmental toxicity study data in laboratory animals from FDA files or directly from pharmaceutical companies on the same products; 3. Extract specific data elements into a database for qualitative and quantitative comparison; 4. Evaluate data using the expertise of pharmacology/toxicology and clinical/epidemiology project participants; 5. Conduct statistical analyses, initially using multiple regression analyses and correlation approaches, with more sophisticated analyses as the data permit; and 6. Draw conclusions about the predictability of animal testing data, and recommend design improvements as appropriate.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0703601	**Hansen, D.** Chen, J.J. Ferguson Laborde Wilkes	In Review/ **Repro Lab/** AGNT	An Investigation of the Possible Developmental Toxicity of St. John's Wort (Hypericin)	1. Determine if administration of St. John's Wort by gavage alters embryonic growth and development in rats; 2. Determine if maternal administration of St. John's Wort to rats prenatally and during the first three weeks after birth alters early postnatal growth and survival; 3. Determine if maternal administration of St. John's Wort to rats prenatally and during the first three weeks after birth alters learning and various behaviors later in life.
E0703701	**Delongchamp** Chen, J.J. Lang Lung-An	In Review/ **Biometry** METH	A Mixture Model Approach to Classifying CYP1A2 Variants that Adjusts for their Current Smoking Status	1. Examine statistical methods for parametric density estima-tion based upon a mixture of normal distributions; 2. Apply the method to a data set where hepatic cytochrome P4501A2 activity appears to be induced by smoking cigarettes.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0703801	**Ali** Duhart DunkelIman Lipe Newport Schmued Schnellmann Slikker Whittaker	In Review/ **Neuro Tox/** AGNT	Acute toxicity on iron compounds in young mice and rats (CFSAN)	1. Compare acute toxicity in young animals using two forms of iron commonly used in iron supplements and one form that is to be used in fortification. 2. Determine if acute high doses of iron compounds produce reactive/ oxygen species, and alteration in the lipid peroxidation and changes in antioxidant enzymes in different regions of brain and liver of young mice and rat. 3. Determine the effect of acute high doses of iron compounds on CBC, MCV, MCHC, TIBC and the distribution of iron and iron-binding proteins in different regions of brain and other visceral organs in young animals. 4. Determine if acute high doses of iron compounds produce significant changes in neurotransmitter concentrations and activity of nitric oxide synthase in different regions of brain in young mice and rats 5. Determine if acute high doses of iron compounds produce pathological alteration in brain and other visceral organs in young mice.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0704001	**Ferguson** Ali Gough Paule	Active/ **Neuro Tox/** CNPT	Validity of Developmental Cerebellar Stunting in the Rat as a Model for Attention Deficit Hyperactivity Disorder: Behavior and Neurochemistry	1. Identify treatments which cause developmental cerebellar stunting, specifically those which decrease the granule cell population with few effects on Purkinje cells. 2. Confirm the increase in locomotor activity caused by developmental cerebellar stunting and to determine the degree to which this hyperactivity resembles human ADHD. 3. Identify other behavioral alterations associated with developmental cerebellar stunting and to determine the degree to which these resemble those associated with human ADHD. 4. Identify the neurochemical alterations in different brain regions resulting from the developmental insult. 5. Compare these neurobehavioral and neurochemical alterations to those exhibited by the most common rodent model of ADHD: the Spontaneously Hypertensive Rat (SHR).
E0704101	**Parsons** Heflich	Active/ **Gen Lab/** PRED	Measurement of H-*ras* Codon 61 CAA AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection	1. Quantify somatic mutations in liver DNA of mice treated with 4-aminobiphenyl in order to establish and evaluate MutEx/ACB-PCR genotypic selection as an approach for human risk assessment. 2. Determine whether or not the MutEx/ACB-PCR genotypic selection is sensitive enough to measure the spontaneous frequencies of H-*ras* codon 61 CAA AAA mutation in three different mouse models: B6C3F1, C57BL/6, and the Pms2 mismatch repair-deficient, transgenic mouse.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0704401	**Chelonis** Paule	In Review/ **Neuro Tox/** AGNT	Complex Brain Function in Autistic Children	Compare brain functioning among autistic children and normal functioning children using tests that assess motivation, color/position discrimination and memory. Additionally, these measures will be compared among autistic children with various degrees of symptom severity.
E0704501	**Kang** Chen, J.J. Kodell	In Review/ **Biometry/** PRED	Dose-Response Modeling for Microbial Risk Assessment	1. Evaluate existing dose-response models for microbial risk assessment. 2. Develop improved models for estimating probabilities of infection and disease. 3. Develop methods for incorporating model uncertainty into microbial risk assessment.

Project Number	**Principal/** Co-Principal Investigator(s)	Status/ **Res. Area/** GOAL	Title	Objective
E0704701	**Harris** Casciano	In Review/ **Gen Lab/** CNPT	Modulation of Gene Expression in Chemical Carcinogenesis: Analysis of Aflatoxin B1 Induced Gene Expression in Human Hepatocytes	1. Evaluate the expression patterns of eight genes previously identified by differential hybridization of a gene filter array to be aflatoxin B1 (AFB1)-responsive in human hepatocytes. Use Northern blot, RT-PCR and/or RNA protection assay to establish an AFB1-dose response curve of gene induction and determine the minimum dose at which gene induction can be detected for each gene. 2. Identify additional AFB1 induced genes using differential display PCR (DD-PCR) and differential hybridization of a high density filter array utilizing mRNA from human hepatocytes treated with low, moderate and cytotoxic levels of AFB1. Evaluate selected genes as described for objective #1. 3. Determine the role of reactive/ oxygen species and signal transduction pathways (protein phosphorylation patterns) in the induction of selected AFB1-induced genes. 4. Analyze protein expression by Western blot analysis of selected AFB1-induced genes. 5. Compare gene induction in rat and human hepatocytes, and in human hepatocytes ager treatment with other chemicals (selected genes) using Northern blot analysis.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0704801	**Khan** Cerniglia Gilbert Jones Miller Nawaz Summage-West	In Review/ **Micro/** METH	Studies on Mechanism of Fluoroquinolones Resistant *Salmonella spp*. Isolated from Animal Feeds (Poultry), Animal production Environment and the Development of Molecular Methods for Screening the Drug Resistance Genes (CVM)	1. Isolation, identification and characterization of nalidixic acid and fluoroquinolone resistant *Salmonella spp*. from chicken farms (animal feed, feces, manure, litters and animals) by biochemical and Polymerase Chain Reaction. 2. Determination of minimum inhibitory concentration for environmental isolates, development of molecular techniques and its comparison with clinical strains. 3. Determination of drug resistance mechanisms in the environmental isolates and their characterization by molecular techniques, i.e., plasmid analysis, complementation analysis for gyrB gene mutation, amplification of resistance gene by PCR and restriction analysis and single-stranded conformational polymorphism analysis for mutation on gyrA and gyrB gene. 4. Determination of influence of seasons and the frequency of isolation of fluoroquinolone-resistant *Salmonella spp*.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0704901	**Wagner** Cerniglia Holland Jones	In Review/ **Micro/** METH	*In Vitro* Model and Molecular Analysis of Competitive Exclusion Products (CVM)	1. Evaluate individual component bacteria in a defined competitive exclusion (CE) product for exclusion of enteric pathogens from Caco-2 cell monolayers; 2. Define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements; 3. Sequence analysis of 16s rRNA polymerase chain reaction (PCR) products from defined culture component bacteria and development of a data base containing the sequences for use in subsequent identification of the organisms in undefined CE products; 4. Application of the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.
E0705001	**Nawaz** Cerniglia Gilbert Khan Jones Miller Pothuluri Steele	In Review/ **Micro/** METH	Studies on the Fluoroquinolone Resistance in *Campylobacter sp*. Isolated from Poultry (CVM)	1. Isolation, identification and characterization of fluoroquinolone-resistant *Campylobacter sp*., from water, feed samples in poultry houses and from litter; 2. Determination of the optimum concentration of nalidixic acid and fluoroquinolonene resistance in different members of Campylobacter spp.; 3. Molecular characterization of fluoroquinolone resistance by polymerase chain reaction (PCR), Nucleotide sequencing and Single-strand conformation polymorphism (SSCP); 4. Determination of the influence of various seasons and the frequency of isolation of fluoroquinolonene-resistant *Campylobacter spp*.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
E0705101	**Wang** Cao Cerniglia	In Review/ **Micro/** METH	Performance Evaluation of the FDA Bacteriological Analytical Manual (BAM) Cultural and Molecular Methods to Identify and Quantitate Human Foodborne Pathogens in Animals Feeds, the Animal Production Environment.	1. Development and testing of rapid sampling strategies for use in animal feeds, feces, and human foods; 2. Comparing different sample preparation methods with our "universal polymerase chain reaction (PCR) protocol"; 3. Performance evaluation of the FDA bacteriological analytical manual (BAM) cultural methods with our "universal PCR protocol"; 4. Optimization of the methods found not to perform satisfactorily; 5. Preparation of a routine PCR protocol for rapid detection and identification of human foodborne pathogens in animals feeds, the animal production environment including feces, and human foods.
E0705201	**Sutherland** Cerniglia Freeman Lay Parshiko Williams	In Review/ **Micro/** KNLG	Microbial Models for Biotransformation of Fluoroquinolones	Develop a microbial model of fluoroquinolone drug transformation. The hypothesis of the research is that microorganisms will be found that have the ability to biotransform fluorquinolones to products that are relevant to mammalian studies.
E0705401	**Griffin** Brand Kadlubar	In Review/ **Tech Adv/** AGNT	Potassium Permanganate Target Animal Safety	Determine the safety of 36 hour exposures of channel catfish and rainbow trout to three concentrations of potassium permanganate.
P00268	**Flammang** Casciano Chen, J.J. Kodell	Active/ **OR/Imm Off** CTR SUP R	NCTR and FDA *Integrated Research*	Interaction with and development of collaborative projects with other FDA Centers.

Project Number	**Principal/** Co-Principal Investigator(s)	Status/ **Res. Area/** GOAL	Title	Objective
P00380	**Tolleson**	Completed/ **Bio Tox/** METH	Development of *In Vitro* Human Cell Culture Systems to Screen Compounds Suspected to have Estrogenic or Anti-Estrogenic Activity (OWH)	The mission of the FDA is to protect the health of Americans. As a result, the FDA strives to ensure that foods and drugs are safe and effective for their intended purposes. The success of the FDA mission depends on the development of improved techniques for the assessment of risk to human health. We recently submitted an application to the FDA Office of Women's Health (OWH) that proposed to develop alternative facile and economical cell biological approaches to determine the role of human cytochromes P-450, UDP-glucuronosyltransferases, and sulfotransferases in the etiology of endocrine disrupting compounds The aim of the research project presented here is to obtain preliminary results to support the OWH proposal. The study that we present here is designed to generate the molecular biological systems necessary to begin evaluating the role of human cytochromes P-450, UDP-glucuronosyltransferases, and sulfotransferases in modulating the estrogenic nature of putative endocrine disrupting agents.
P00383	**Lomax**	Completed/ **Pathology/** CTR SUP R	Data Extraction for Urinary Bladder	Scan the Pathology database for neoplastic morphologies for urinary bladders for all strains of mice. Specific data to be presented to ILSI Risk Science Institute, and the Registry of Toxicologic Pathology for Animals. Project will require services of computer staff (ROW).

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
P00386	**Paule**	Active/ **Neuro Tox/** PRED	Arkansas Children's Hospital Statistical Support	Project will involve an empirical investigation of OTB performance by normal children and children identified as expressing specific clinical diagnoses including Attention Deficit Disorder with or without Hyperactivity.
P00388	**Streck**	Active/ **Repro Lab/** PRED	Identification of Molecular Markers of Peroxisome Proliferator-Activated Receptor-Gamma Activation in the Rat Fetus	1. Determine which rat fetal tissues express peroxisome proliferator-activated receptor (PPARY). 2. Identify, from a set of genes regulated by PPARY in adults, genes that are coexpressed in the same fetal tissues as express PPARY.
P00390	**Howard** Melchior	Completed/ **Bio Tox/** PRED	Detection of Toxins in Lake DeGray Coots Using a Mouse Bioassay	Determine if a "mouse bioassay for neurotoxins" can detect the Lake DeGray toxins in either (1) the coots or (2) the vegetative matter that is part of the coot diet.
P00393	**Young**	Active/ **Biometry/** PRED	Species Comparison Utilizing a PBPK Model	Pharmacokinetic data from the literature will be excerpted and adapted to be simulated via a PBPK model. Initially the literature data will be limited to dexamethasone, cocaine, and methylmercury. Species comparisons will be made utilizing this single pharmacokinetic model.
P00396	**Dalu**	Completed/ **Bio Tox/** AGNT	Methods Development for the Analysis of the Effects of Genistein on Estrogen and Growth Factor Receptor-mediated Signaling Pathways in Mammary Gland and Uterus	To utilize mammary and uterine tissue, which would otherwise be discarded, from the carcasses of the female pups of E02122.13 for methods development to evaluate the molecular mechanisms of the modulation of mammary carcinogenesis by the soy isoflavone genistein.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
P00397	**Parsons**	Active/ **Gen Lab/** METH	Enrichment of *ras* Gene Sequence Through Hybrid Selection	The livers of these animals will be used to isolate genomic DNA and this DNA will be used to develop a hybridization method for the gene-specific enrichment of the *ras* oncogene. The outcome of this experiment will determine how many animals will be required in a planned protocol on the uses of genotypic selection methods to measure chemically induced *ras* mutations.
P00398	**Sheehan** Branham Vom Saal	Active/ **Repro Lab/** KNLG	Experimental Assessment of Environmental Estrogens - Contract with University of Missouri	Quantitate the major potency determinants of two estrogenic chemicals: genistein, a naturally occurring plant hormone, and methoxychlor, a persistent chlorinated environmental chemical.
P00404	**Binienda**	Active/ **Neuro Tox/** CNPT	Development of Electroencephalography Data Acquisition System in the Conscious Rat	Develop and test a system for EEG data acquisition and analysis in the conscious rat we will apply the implantation procedure to allow direct connection of the implanted electrodes in conscious animals with the signal amplifiers. Surgical preparation of the electrodes and connectors will be followed by recording of the EEG signals after a routine recovery period (7 days).
P00405	**Howard** Couch Doerge Melchior Scallet	In Review/ **Bio Tox/** CNPT	Identification of the CEBLS Toxin in Lake DeGray	1. Determine if a correlation exists between vacuolar myelinopathy in the coots and toxicity of coot organ extracts following injection into mice, 2. Isolate and identify the causative agent; 3. Establish a method for toxin detection; 4. Provide interested investigators (e.g., NCTR Division of Neurotoxicology) with enriched or purified toxin for determination of the mechanism of action of toxicity in rodents or birds.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
P00406	**Lu** Tang	Active/ **Repro Lab/** KNLG	Cellular Effects of Endocrine Disrupting Chemicals in Rats Evaluated by Flow Cytometric Cell Cycle Analysis	1. Establish a working procedure using flow cytometric cell cycle analysis in our laboratory to evaluate uterotrophic and ovarian effects of endocrine disruptors in rats to provide data for the program of Estrogen Knowledge Base at NCTR; 2. Efficiently utilize the available tissues without requesting the additional rats to obtain extra endpoint information to be used in the NTP studies at the NCTR
S00031	**Warbritton**	Active/ **Pathology/** METH	Immunohistochemistry Methods Development	New experiment methods development for which no number has been assigned.
S00138	**Paule**	Active/ **Neuro Tox/** METH	Nonhuman Primate Operant Behavior Training and Maintenance	Produce and maintain trained animals in NCTR's Operant Test Battery. Animals are primarily Rhesus monkeys. No experimental manipulations such as drug exposure will occur in any subjects under this project number.
S00143	**Duffy** Ali Feuers	Active/ **Repro Lab/** METH	Development of an Automated Repetitive Blood Sampling System to Measure Circadian Rhythms of Blood Chemistries	Train investigators in IV cannulation techniques which will reduce the number of animals needed to determine circadian rhythms of blood consituents; develop IV and IG infusion methods to administer drugs and food in a more controlled manner; to develop an automated repetitive small volume sampling system.
S00162	**Laborde**	Active/ **Repro Lab/** METH	Teratology Training	Train researchers in the techniques of teratological evaluation of rat and/or mouse fetuses.
S00163	**Hansen, D.**	Active/ **Repro Lab/** PRED	Embryo Culture Training	Train researchers in the technique of rodent whole embryo culture using mouse and/or rat fetuses.
S00170	**Rafii**	Active/ **Micro/** CTR SUP R	Animals for Antibody Production	Provide antibodies for microbiology program research efforts.

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S00173	**Gillam**	Active/ **Neuro Tox/** METH	Procedure for Ambulation Exercise of Nonhuman Primates Using the Controlled Ambulation Device (CAD)	Provide training of nonhuman primates in the CAD apparatus.
S00174	**Kodell** Alderson Chen, J.J. Collins Jacobson Pohland	Active/ **Biometry/** METH	Modification and Application of Quantitative Risk Assessment Techniques to FDA Regulated Products (CFSAN)	In response to requests from scientists and regulators at CDRH, CDER, CFSAN, and CVM, using available toxicological data, conduct cancer and noncancer risk assessments of FDA regulated products to assist in establishing "safest" conditions of exposure to toxic substance.
S00175	**Kodell** Chen, J.J.	Active/ **Biometry/** CNPT	Application of Biometrical Procedures for NTP Projects	In response to requests from NCTR scientists, modify and/ or apply statistical techniques to the design, conduct, analysis, and interpretation of NTP studies to identify and assess the cancer and noncancer risks of potentially toxic substances.
S00179	**Smith** Beland McGregor	Active/ **Bio Tox/** AGNT	*Salmonella* Mutagenicity Testing for Regulatory Needs (CDER)	Use the Ames *Salmonella* mutagenicity test system to determine the mutagenicity of compounds of regulatory interest to the Agency.
S00185	**Campbell**	Active/ **Micro/** CTR SUP R	Special Epidemiology Investigations of Potential Microbiological Contamination Problems	Investigate potential microbiological contamination problems. To report non-routine sample time which is not recorded on Sample Collection Report (SCR).
S00189	**Holland** Chamberland	Active/ **Micro/** METH	Tuberculocidal Efficiency of Various Disinfectants (CDRH)	This support number established to take the place of E06793.00 - Assess, modify and validate the AOAC tuberculocidal test procedures for use with disinfectants.
S00197	**Lay**	Active/ **Chem/** METH	Chemistry Support for ORA's Jefferson Regional Laboratory's TCDD Analysis Program	FDA's needs for the analysis of Dioxins (TCDD) in a timely manner requires assistance from the Division of Chemistry.

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S00198	**Beland**	Active/ **Bio Tox/** AGNT	*In vivo* DNA Adduct Standards	This Support number is being set up to take the place of P00371 - Per division, this will be an ongoing support number in collaboration with IARC. - P00371 has been closed out.
S00205	**Howard** Couch	In Review/ **Bio Tox/** METH	Synthesis of gamma [32P] - ATP for 32P-postlabeling	To cover the personnel time for the synthesis of γ[32P] - ATP. This material is synthesized in support of the needs of the Division of Biochemical Toxicology. Several investigators in the Division use carrier-free γ[32P]ATP in the detection of DNA adducts using the 32P-postlabeling method. The synthesis involves order 32P-phosphate (carrier-free) from ICN Radiopharmaceuticals, Inc., and enzymatically converting the 32P-phosphate to γ[32P]ATP. The γ[32P}ATP is then transferred to authorized investigators on an "as need" basis. The advantages of on-site γ[32P]ATP synthesis are twofold: 1. The cost is less than one-half of the cost of purchasing γ[32P]ATP; 2. The number of shipments of radioisotopes to the Center is considerably decreased. We have performed this service for the last 5 years. This request is to cover the personnel time required for the γ[32P]ATP synthesis and distribution, and maintenance of radiation safety records.
S00208	**Casciano**	Active/ **Gen Lab/** CTR SUP R	Evaluations of Genotoxicity in Specific Compounds in Response to Solicitations by Colleagues in Various Product Centers	Project number set up to track resources from Genetic Toxicology that are used for responding to solicitations by our colleagues in various product centers to evaluate the genotoxicity of a specific compound.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
X30029	**Slikker** Gaylor Sobotka	Proposed/ **Neuro Tox/** METH	Risk Assessment of Neuro-toxicants	Project under development.
X60021	**Shaddock** Pipkin	Proposed/ **Gen Lab/** CNPT	A Study of Several Hepato-carcinogens in the Rat Hepatocyte System: The Effect of Aflatoxin B1 (AFB1), 2-AAF, Clofibrate and Metha-pyrilene on Gene Expression and Induction of Stress Proteins	Project under development.
X60043	**Rowland** Paule	Proposed/ **Neuro Tox/** CNPT	Risk Factors for Attention-Deficit/Hyperactivity Disorder (ADHD)	This project will involve an epidemiologic study of several possible environmental risk factors associated with the occurrence of ADHD in a large population of school age children (grades 1-5). Com-ponents of the NCTR Operant Test Battery will be used to assist in the clinical assess-ment of ADHD status.
X70004	**Streck** Webb	Proposed/ **Repro Lab/** PRED	Retinoic Acid Receptor Expression	Project under development.
X70010	**Howard**	Proposed/ **Bio Tox/** CNPT	Characterization and Expres-sion of Fumonisin Binding Proteins	Project under development.
X70026	**Manjanatha**	Proposed/ **Gen Lab/** PRED	A Study of DNA Repair in the Transgene of Big Blue Rats	Project under development.

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X70033	**Shuttleworth** Cerniglia Hansen, E.	Proposed/ **Micro/** PRED	Effects of Physico-Chemical Factors on the Metabolic Potential of the Drug-Metabolizing Fungus, *Cunninghamella elegans*.	Determine how various physico-chemical factors affect the drug-metabolizing capacity of the fungus *Cunninghamella elegans*. This fungus has been used as a microbial model for the eukaryotic metabolism of various drugs of pharmacological interest; however, the interrelationship between general fungal physiology and drug metabolism has not been investigated. In this study, we will gain a better understanding of that interrelationship so that we can enhance the production of drug metabolites of interest.
X70044	**Kodell** Doerge	Proposed/ **Biometry/** METH	Statistical Evaluation of Mass Spec Confirmation Methods for Regulation Purposes	Project under development.
X70045	**Kodell** George	Proposed/ **Biometry/** METH	Trend Test for Clustered Exchangeable Binary Data	Project under development.
X70048	**Kodell** Chen, J.J. Gaylor Zheng	Proposed/ **Biometry/** KNLG	A New Strategy for Detecting Carcinogenicity	Project under development.
X70049	**Sutherland** Castleberry Cerniglia Freeman Holcomb Williams	Proposed/ **Micro/** AGNT	Methods for Detection of *Beauvericin* and *Moniliformin*	Development of HPLC and capillary electrophoresis methods for detection of the Fusarium mycotoxins, *beauvericin* and *moniliformin* in foods.
X70053	**Turturro**	Proposed/ **Biometry/** AGNT	Model Toxic Response Using Neural Networks	Project under development.
X70059	**Lu**	Proposed/ **Repro Lab/** AGNT	Investigation of Reproductive and Developmental Toxicity of Tamoxifen by Flow Cytometric Cell Cycle Analysis	Project under development.
X80004	**Roberts**	Proposed/ **Bio Tox/** CNPT	Immunochemical Evaluation of Oxidative Damage and Autoimmunity	Project under development.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
X80005	**Delclos**	Proposed/ **Bio Tox/** AGNT	The Distribution and Metabolism of Genistein in CD Rats	Project under development.
X80006	**Delclos** Blaydes Dalu	Proposed/ **Bio Tox/** AGNT	Strain Differences and the Influence of Leachable Estrogenic Substances on the Responses to Implanted Materials	Project under development.
X80007	**Manjanatha**	Proposed/ **Gen Lab/** AGNT	Evaluation of Chemicals of Interest to the FDA/NCTR in the Transgenic *lacL* Rat2 Cell line	Project under development.
X80012	**McClure** Ambrosone Fu Kadlubar	Proposed/ **Mol Epi/** PRED	Quantification of Chloral Hydrate Induced Lipid Peroxidation in Children	Project under development.
X80021	**Delongchamp** Kadlubar Lang	Proposed/ **Biometry/** METH	An Investigation of Mode Tree Methods	Project under development.
X80023	**Poirier** Wise	Proposed/ **Mol Epi/** PRED	Homocystine Toxicity	Project under development.
X80024	**Poirier** Wise	Proposed/ **Mol Epi/** CNPT	Methyltransferase in Human Colon Cancer	Extend to humans, findings made with liver tumors in methyl-deficient rats.
X80025	**Scallet**	Proposed/ **Neuro Tox/** METH	Development of an Assay for HACCP Regulation of Transmissible Spongioform Encephalopathies	Project under development.
X80031	**Tang** Kadlubar	Proposed/ **Mol Epi/** PRED	Breast Cancer and the Genetic Polymorphism of Estrogen Sulfotransferase	Human estrogen sulfotransferase is a newly identified gene and is found involved in the metabolism of estrogens, oral contraceptives and anti-estrogens such as tamoxifen, which is used in breast cancer chemotherapy. The results of this study will be helpful to predict breast cancer risk and to assist clinicians to determine the breast cancer chemotherapeutic strategies for each patient.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
X80033	**Kadlubar** Ambrosone Thompson-Carino	Proposed/ **Mol Epi/** PRED	Rapid, Population-based, Screening Methodology for Genetic Polymorphisms in Adverse Drug Metabolizing and/or Cancer-Related Risk Alleles (CRADA)	1. Development and fabrication of bioarray chip or "risk chip" for the analysis of genetic polymorphisms that affect individual cancer or adverse drug risk; 2. Validation of "risk chip" by comparative analyses with standardized methodologies; 3. Automation of methodologies for large population risk assessment using "risk chip" in a robotic workstation; 4. Establish a PHS laboratory at NCTR as an alpha test site to introduce "risk chip;' screening analysis as rapid and reliable frontline screening methodology for clinical and population-based molecular epidemiological studies.
X80038	**Chelonis** Paule	Proposed/ **Neuro Tox/** AGNT	Assessment of Decision Making	Assess impulsivity and procrastination in children with various psychological and physiological problems, and their parents.
X90001	**Sheehan** Fang Perkins Tong	Proposed/ **Repro Lab/** KNLG	Computational Chemistry Models to Determine the Structural Characteristics of Chemicals that are Known Estrogen Receptor Antagonists	Develop computational chemistry models to determine the structural characteristics of chemicals that are known estrogen receptor antagonists, and to use this information to design other such antagonists that have different characteristics of potential use in treating breast cancer.
X90002	**Young** Pearce	Proposed/ **Biometry/** PRED	Models for Bacterial Growth and Death	Project under development.
X90003	**Turturro**	Proposed/ **Biometry/** PRED	Predictive Toxicology	Project under development.
X90004	**Lay** Holder Holland Musser Voorhees	Proposed/ **Chem/** KNLG	Characterizing Bacterial Proteins by M.S.	Project under development.

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X90005	**Beger**	Proposed/ **Chem/** METH	NMR of DNA and Adducts	Project under development.
X90006	**Leakey**	Proposed/ **Chem/** METH	Toxicology of Herbal Products	Project under development.
X90007	**Ang**	Proposed/ **Chem/** METH	Chemical and Biological Characteristics of Medicinal Botanicals	Project under development.
X90009	**Khan** Cerniglia Cha Robertson	Proposed/ **Micro/** METH	Studies on Mechanism of Malachite Green Degradation by Anaerobic Bacteria	Project under development.
X90011	**Khan** Cerniglia	Proposed/ **Micro/** METH	Studies on Mechanism of Multiple Drug Resistant *Salmonella typhimurium* DT104	Project under development.
X90013	**Pothuluri** Cerniglia Pak	Proposed/ **Micro/** METH	Microbial Degradation of Drugs and Feed Additives used in Fish Farming (Aquaculture)	Project under development.
X90014	**Pothuluri** Cerniglia Laurenzana	Proposed/ **Micro/** METH	Fungal Metabolism of Agricultural Pesticides, Vinclozolin and Methoxychlor, found in Food Products	Project under development.
X90015	**Ali** Duhart Klein Lipe Newpor Slikker	Proposed/ **Neuro Tox/** METH	Neurotoxicity of GHB and Butyolactone	Project under development.
X90016	**Ali** DuhartItzhak Mukherjee Newport Slikker Yu	Proposed/ **Neuro Tox/** KNLG	The Role of NOS and NFKB in Dopamine Neurotoxicity Induced by methamphetamine (METH) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)	Project under development.
X90023	**Doerge** Churchwell	Proposed/ **Bio Tox/** AGNT	Soy/Infants	Project under development.
X90024	**Roberts**	Proposed/ **Bio Tox/** METH	Antigenic Biomarkers of Estrogen Catechol Metabolism for Use in Epidemiological Studies	Project under development.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
X90025	**Shelton** Manjanatha	Proposed/ **Gen Lab/** PRED	Molecular Analysis of C11 Mutations in Rat 2 Cells Exposed to Several Mutagenic Carcinogens	Project under development.
X90026	**Manjanatha** Shelton	Proposed/ **Gen Lab/** PRED	A Study of the Repair Properties of P53 Deficient Animal Models	Project under development.
X90027	**Dobrovolsky** Heflich	Proposed/ **Gen Lab/** PRED	A Novel Transgenic Model for Mutation Detection Using Fluorescent Markers	Project under development.
X90028	**Morris**	Proposed/ **Gen Lab/** PRED	Tumorigenic Effect of Genistein in the Model P53 Deficient Mouse	Project under development.
X90029	**McGarrity** Domon Morris	Proposed/ **Gen Lab/** AGNT	Suppression of Apoptosis by Ameloride and the Effect on the Mutant Frequency in Lymphoblastoid Cells	Project under development.
X90030	**Morris**	Proposed/ **Gen Lab/** PRED	Molecular Characterization of Thymidine Kinase Mutants	Project under development.
X90031	**Domon** Bishop McGarrity Morris	Proposed/ **Gen Lab/** AGNT	An Evaluation of the Genotoxicity of Coumestrol	Project under development.
X90032	**Valentine**	Proposed/ **Gen Lab/** PRED	Development of a Transgenic Cell Culture System to Detect Microsattelite Instability	Project under development.
X90033	**Streck** Webb	Proposed/ **Repro Lab/** PRED	Growth Factor Treatment of Cultured Limbs	Project under development.
X90044	**Hass**	Proposed/ **Repro Lab/** AGNT	Evaluation of Phototoxicity in Several *In Vitro* and *In Vivo* Models	Project under development.
X900477	**Blair**	Proposed/ **Repro Lab/** KNLG	EDSTAT Standardization and Validation of Uterotropic Assays	Project under development.
X90048	**Branham** Dial Moland	Proposed/ **Repro Lab/** KNLG	Prenatal Genistein Non-Monotoxic Dose Response Curves	Project under development.
X90049	**Sheehan** Branham	Proposed/ **Repro Lab/** KNLG	An Evaluation of Gene Expression Using an Estrogen Gene Chip	Project under development.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ GOAL	Title	Objective
X90050	**Sheehan** Blair Dial Moland	Proposed/ **Repro Lab/** KNLG	Improved Methods for Follicle Counts	Project under development.
X90051	**Sheehan** Branham Fang	Proposed/ **Repro Lab/** KNLG	Do Thresholds Exist for Uterotropic Response for Estrogens	Project under development.

INTRA-AGENCY FUNDED PROJECTS

INTRA-AGENCY FUNDED PROJECTS

Cooperating Organization: Center for Veterinary Medicine(Transfer Funds)

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0704801	**Khan** Nawaz Summage-West Gilbert Jones Cerniglia Miller Khan	In Review/ **Micro**/ METH	Studies on Mechanism of Fluoroquinolones Resistant *Salmonella spp*. Isolated from Animal Feeds (Poultry), Animal Production Environment and the Development of Molecular Methods for Screening the Drug Resistance Genes (CVM)	1. Isolation, identification and characterization of nalidixic acid and fluoroquinolone resistant *Salmonella spp*. from chicken farms (animal feed, feces, manure, litters and animala) by biochemical and Polymerase Chain Reaction. 2. Determination of minimum inhibitory concentration for environmental isolates, development of molecular techniques and its comparison with clinical strains. 3. Determination of drug resistance mechanisms in the environmental isolates and their characterization by molecular techniques, i.e., plasmid analysis, complementation analysis for gyrB gene mutation, amplification of resistance gene by polymerase chain reaction (PCR) and restriction analysis and single-stranded conformational polymorphism analysis for mutation on gyrA and gyrB gene. 4. Determination of influence of seasons and the frequency of isolation of fluoroquinolone-resistant *Salmonella spp.*

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0704901	**Wagner** Cerniglia Holland Jones	In Review/ **Micro/** METH	*In Vitro* Model and Molecular Analysis of Competitive Exclusion Products (CVM)	1. Evaluate individual component bacteria in a defined competitive exclusion (CE) product for exclusion of enteric pathogens from Caco-2 cell monolayers; 2. Define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements; 3. Sequence analysis of 16s rRNA polymerase chain reaction (PCR) products from defined culture component bacteria and development of a data base containing the sequences for use in subsequent identification of the organisms in undefined CE products; 4. Application of the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.
E0705001	**Nawaz** Cerniglia Gilbert Jones Khan, A. Khan, S. Miller Pothuluri Steele	In Review/ **Micro/** METH	Studies on the Fluoroquinolone Resistance in *Campylobacter sp.* Isolated from Poultry (CVM)	1. Isolation, identification and characterization of fluoroquinolone-resistant *Campylobacter sp*., from water, feed samples in poultry houses and from litter; 2. Determination of the optimum concentration of nalidixic acid and fluoroquinolonene resistance in different members of *Campylobacter spp*.; 3. Molecular characterization of fluoroquinolone resistance by PCR, Nucleotide sequencing and Single-strand conformation polymorphism (SSCP); 4. Determination of the influence of various seasons and the frequency of isolation of fluoroquinolonine-resistant *Campylobacter spp*.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0705301	**Khan, S.** Cerniglia Jones Khan, A. Nawaz	In Review/ **Micro/** METH	Molecular Screening Methods for the Determination of Vancomycin Resistance in Selective Competitive Exclusion Product CF3 (PREEMPT) Bacteria	1. Islolation, identification and biochemical characterization of vancomycin resistant bacteria present in a commercially available competitive exclusion product CF3; 2. Development of a rapid polymerase chain reaction (PCR) method of the detection of vancomycin resistance determinant genes, namely, the Van A0, Van B, Van C and D-ala-D-lac ligase gene Ddl.; 3. The characterization of plasmid DNA Profile and plasmid-mediated drug resistance transfer; 4. Genetic fingerprinting of the vancomycin resistant microorganisms present in PREEMPT culture; 5. Nucleotide sequence analysis of the PCR products of vancomycin resistant determinant genes showing interesting restriction profiles.

Cooperating Organization: Office of Women's Health

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0696101 E0696111	**James** Miller Pogribna	Active/ **Bio Tox/** AGNT	Mechanisms of Immunotoxicity and Carcinogenicity Associated with Silicone Breast Implants	Examine the acute and chronic cellular and subcellular responses to subcutaneous silicone implants utilizing state-of-the-art immunohistochemistry and molecular biology technologies.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0697301	**Streck** Branham Sheehan	Active/ **Repro Lab/** KNLG	Mechanism of Tamoxifen Developmental Toxicity and Neoplasia: Tamoxifen Effects on the Rat Uterine Insulin Like Growth Factor System	1. Define the ontogeny of insulin-like growth factor (IGF) system mRNA expression in the developing rat uterus. 2. Determine the uterine cell types in which IGF system mRNAs are expressed; 3. Determine the effects of diethylstilbestrol (DES), tamoxifen (TAM) and ICI 182,780 on IGF system mRNA expression at selected developmental stages.
E0700801	**Sheehan** Branham Hass	Active/ **Repro Lab/** AGNT	Bioassay of Reproductive Tract Toxicities Caused by Genistein and Methoxychlor in Sprague-Dawley Rats	Perform a battery of analyses, which will determine the capacity of two xenoestrogens, genistein and methoxychlor, administered via two exposure routes to induce estrogen responses and reproductive tract developmental toxicities in rodents. This objective covers the ovary and uterus. A continuous feeding protocol will be compared to a 5-day injection protocol at 4 periods of development.
E0701101	**Beland** Marques	Active/ **Bio Tox/** AGNT	DNA Adducts of Tamoxifen	The nonsteroidal antiestrogen tamoxifen, which is currently being used in clinical trials as a chemoprotective agent against breast cancer, has been associated with the induction of certain malignancies. In order to determine if tamoxifen is acting through a genotoxic mechanism, this project will characterize DNA adducts from suspected tamoxifen metabolites, and develop methods for their detection and quantitation.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0701501	**Ambrosone** Stone Thompson-carino	Active/ Mol Epid/ METH	Breast Cancer in African-American Women: Metabolic Modification of Dietary and Hormonal Risk Factors	Examine the role of inter-individual variability in response to exogenous agents as it may relate to breast cancer risk in African-American women. By evaluating risk associated with exposure to oral contraceptives, hormone replacement therapy, and modification of that risk by genetic variability in their metabolism, the effects of substances regulated by the FDA on breast cancer risk in African-American women may be further elucidated. Additionally, a successful model to increase African-American participation in research studies would greatly assist in future studies related to FDA regulated substances in African-American populations.
E0701601	**James** Ames Gibson Hine	Active/ **Bio Tox/** CNPT	Molecular and Metabolic Determinants of Maternal Risk and Progression of Down Syndrome: Potential for Nutritional Interventions (OWH funded project)	1. Define abnormalities in one-carbon metabolism in mitogen-stimulated lymphocytes from women who have had a child with Down Syndrome and to determine whether appropriate folate/ methyl supplementation can normalize these metabolic abnormalities; 2. Define the biochemical and molecular consequences of abnormal one-carbon metabolism in mitogen-stimulated lymphocytes from Down Syndrome children and to determine whether these metabolic abnormalities can be normalized with targeted nutritional intervention.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0702301	**Tolleson** Howard Jenkins Leakey Morris Rowland	Active/ **Bio Tox/** METH	The Role of Human Metabolism in Endocrine Disruption	Utilize cell biological approaches to determine the role of human cytochromes P-450, UDP-glucuronosyltransferases, and sulfotransferases in the antiestrogens. The relative abilities of the various human enzyme systems expressed by individual cell lines to alter the extent of green fluorescent protein synthesis will indicate those human enzyme activities that activate or deactivate endocrine disrupting agents.
E0704301	**Thompson-carino** Ambrosone Delongchamp Kadlubar Lang	Active/ Mol Epid/ PRED	*In Vivo* Modeling of Steroid-mediated Gender Effects in Drug Metabolism	1. Characterize the activity of CYP1A2 in female subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT; 2. Characterize the activity of CYP1A2 in male subjects with regard to age; 3. Measure estradiol, progesterone, testosterone, cortisol, Il-1, IL-6 and IL-10 levels in female and male subjects studied for CYP1A2 activity; 4. Correlate the activity of CYP1A2 with circulating levels of cytokines and/or circulating levels of steroid hormones; 5. Statistically assess the impact of each of the measured variables on the CYP1A2 phenotype.
P00358	**Sheehan** Branham Burroughs Medlock	Active/ **Repro Lab/** KNLG	Training in the Estrogen Developmental Toxicity Bioassay	Train Pathology Associates, Inc. technicians in animal sacrifice, tissue removal and processing, instrumentation, morphometric techniques, aspects of project management, and procedures for data collection, recording, retrieval, reduction and summarization.

EXTERNALLY FUNDED PROJECTS

EXTERNALLY FUNDED PROJECTS

Alternate Funding Sources

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0701901 E0701911	**Binienda**	Active/ **Neuro Tox/** CNPT	Experimental Autoimmune Prostatitis: Implications for the Prevention and Treatment of Inflammatory and Neoplastic Disorders of the Prostate Gland	1. Induce an experimental autoimmune prostatitis in male rhesus monkeys by immunizing animals with homogenates of monkey prostate gland admixed with Freund's adjuvant; 2. Identify the target proteins of the induced autoimmune prostatitis by using the immune sera (IgG) from animals to screen prostate homogenates by Western immunoblot analyses and screen a monkey prostate cDNA expression library.
X80036	**Chelonis** Blake Paule	Proposed/ **Neuro Tox/** AGNT	Effects of Stimulants on Delay of Gratification	1. Determine the effects of acute administration of several doses of cocaine on the ability of monkeys to delay gratification; 2. Determine the effects of acute administration of several doses of methylphenidate and dextroamphetamine on the ability of monkeys to delay gratification; 3. Generate baseline data for delay of gratification in monkeys.
X90052	**Betz** Ang Lu Yin	Proposed/ **Chem/** CNPT	Biological, Chemical, and Toxicological Investigation of Plant Steroids from Edible Oils	Project under development.

COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENTS

COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENTS

Cooperating Organization: American Cancer Society

Project Number	**Principal/** Co-Principal Investigator(s)	Status/ **Res. Area/** Goal	Title	Objective
E0687501	**James**	Active/ **Bio Tox/** CNPT	Mechanisms of Diet-Induced DNA Damage with Methyl-Donor Deficiency	Further the understanding of the mechanisms by which diet, as an environmental variable, can alter the susceptibility to cancer.

Cooperating Organization: Astra Charnwood

Project Number	**Principal/** Co-Principal Investigator(s)	Status/ **Res. Area/** Goal	Title	Objective
E0280001	**Paule** Binienda Gillam Hammond Pearson Popke Slikker	Active/ **Neuro Tox/** PRED	Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions (97032)- Astra CRADA	1. Establish acquisition curves for several operant behaviors in juvenile rhesus monkeys during chronic oral exposure to two anticonvulsant agents and vehicle; 2. Determine whether exposure results in any significant changes in the acquisition and performance of these operant and other observable behaviors; 3. Determine whether exposure results in any significant changes in clinical chemistry or ophthalmic parameters; 4. Determine plasma distribution profiles and concentrations for each of these agents at various stages of chronic exposure.

Cooperating Organization: Chemical Manufacturers Association

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0290001 X90045	**Sheehan** Blair Branhman Dial Fang Hass Jackson Moland Perkins Purdy Tong	In Review/ **Repro Lab/** KNLG	Development of a Statistically Robust 3D-QSAR Model to Predict *In Vitro* Rat Uterine Estrogen Receptor Binding Activity	Develop and validate a statistically robust model for prediction of isolated rat uterine estrogen receptor relative binding affinity (RBA) that could be used as part of a prioritization scheme to identify chemicals for further *in vitro/in vivo* screening tests.
P00370	**Sheehan** Gaylor Lay Perkins Shvets Strelitz Ulmer	Active/ **Repro Lab/** KNLG	Development of an Estrogen Knowledge Base for Research and Regulation	Identify active elements in estrogen and estrogenic compounds, using the data in the NCTR estrogen database and commercial analysis and modeling tools. The application of traditional and advanced QSAR techniques to this ideal data set should either confirm the existence of active moieties or identify confounding factors that point the way towards further research. In either case, the result of this effort will be an estrogen database with a predictive capability, called a knowledge base.
P00385	**Hass** Branham Sheehan	Active/ **Repro Lab/** KNLG	Validation of the Rat Estrogen Receptor Competitive Binding (RERCB) Assay	Validate the RERCB assay in our laboratory as a basis for providing later data for the Estrogen Knowledge Base

Cooperating Organization: Gentest Corporation

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
X70054	**Leakey** Seng	Proposed/ **Chem/** PRED	Predictive Systems for Human Drug Metabolism	Determine whether age, disease, diet and/or body mass influence expression of hepatic drug metabolizing enzymes in monkeys and humans; to assess what influence altered drug metabolizing enzyme expression will have on drug efficacy and toxicity.

Cooperating Organization: NIH Grant through the Arkansas Children's Hospital

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0703401	**Hansen, D.** Laborde	Active/ **Repro Lab/** CNPT	Indices of Biotin Nutrition	Determine the human requirement for biotin in normal individuals and in individuals in certain circumstances in which biotin status may be impaired. Specific Aim #4 (which will be accomplished at NCTR) will determine whether biotin of similar severity to that observed in human pregnancy can cause significantly increased rates of fetal malformation in the mouse. In the pilot mouse study, marginal biotin deficiency in mouse dams that caused an increase in 3-HIA excretion similar to that seen in human pregnancy produced 100% incidence of cleft palate in the fetal mouse.

INTERAGENCY AGREEMENTS

INTERAGENCY AGREEMENTS

Cooperating Organization: National Institute of Occupational Safety and Health

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0704601	**Wise** Poirier	In Review/ **Mol Epi**/ PRED	Methylation Status and Cancer Risk	Learn whether methylation status, determined by non-invasive procedures, may be a biomarker of cancer risk in humans. The methylation status will be assessed by measurement of SAM, SAH and homocysteine in blood, and of DNA hypomethylation in lymphocytes. Two-thirds of the work will be supported under the terms of an IAG from NCI

Cooperating Organization: National Toxicology Program

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0210601 E0210611	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Chronic Tumor Study of Fumonisin B_1 in Male and Female $B6C3F_1$ Mice (CFSAN)	Determine if dietary fumonisn B_1 is tumorigenic to male and female $B6C3F_1$ mice following chronic dietary exposure.
E0210801 E0210811	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Chronic Tumor Study of Fumonisin B_1 in Male and Female F344 Rats (CFSAN)	Determine the tumorigenicity of fumonisin B_1 in male and female F344 rats following chronic dietary exposure.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0211101 E0211111 E0211121 E0021131 E0021141	**Howard** Binienda Casciano Couch Martinez Melchior Shaddock Slikker Sutherland Tolleson	Active/ **Bio Tox/** AGNT	The Role of Fumonisin B_1 and Other Mycotoxins in *Fusarium* sp. Tumorigenicity in Rats (CVM)	Determine the effect of fumonisin B_1 on signal transduction pathways in cultured human esophageal epithelial tissues. Determine if DNA damage occurs *in vivo* in F344 rats when fed in the diet cultures of *Fusarium graminearum*, *Fusarium subglutinans*, *Fusarium moniliforme* or a combination of the three fungi, using ^{32}P-postlabeling technique. Determine the pharmacokinetics of fumonisin B_1 in $B6C3F_1$ mice and F344 rats under conditions similar to those used in the chronic bioassay, and in nonhuman primates.
E0211301 E0211311 E0211321	**Howard** Dooley Lorentzen Voss	Completed/ **Bio Tox/** AGNT	Sub-chronic (28-day) Study of Fumonisin B_1 in Male and Female $B6C3F_1$ Mice (CFSAN)	Determine the toxicity of fumonisin B_1 in male and female $B6C3F_1$ mice following a 28-day dietary exposure.
E0211601	**Beland** Benson Contrera Gaylor	Active/ **Bio Tox/** AGNT	Tumorigenicity of Chloral Hydrate in $B6C3F_1$ Mice (CDER)	Determine the effect of animal age and duration of exposure upon the tumorigenicity of chloral hydrate in female $B6C3F_1$ mice.
E0211701 E0211711 E0211722	**Leakey** Contrera Seng Turturro	Active/ **Chem/** CNPT	Chronic Bioassay of Chloral Hydrate in Male $B6C3F_1$ Mice Using Idealized Body Weight Curves that are Normalized by Modulation of Caloric Intake (CDER)	Determine the chronic toxicity and potential carcinogenicity of chloral hydrate administered by aqueous gavage, to male $B6C3F_1$ mice. Determine the feasibility of utilizing dietary control (i.e., the manipulation of caloric intake) to control body weight gain so that all mice in each experimental group of the bioassay conform to an idealized weight curve.
E0211801	**Beland** Culp Mulligan	Active/ **Bio Tox/** AGNT	Twenty-eight Day Range Finding Study in Mice and Rats Administered Malachite Green or Leucomalachite Green in the Diet (CVM)	Determine the doses of malachite green to be used in a two-year feeding bioassay and to compare the biological effects from the administration of malachite green and leucomalachite green.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0211901 E0211911	**Doerge** Churchwell Rushing Schmitt	Active/ **Chem/** METH	Development of Analytical Methods for Determination of Malachite Green	1. Develop analytical methods to assess purity of malachite green (MG) and leuco-malachite green (LMG) that will be used in the NTP animal bioassay; 2. Develop analytical methods to quantify MG and LMG content and determine homogeneity and stability in rodent chow under storage and use conditions, ethanol and water for use in the NTP bioassay.
E0212001	**Beland** Benson Chan Lorentzen Roberts	Active/ **Bio Tox/** AGNT	Effect of Ethanol on the Tumorigenicity of Urethane (Ethyl Carbamate) in B6C3F$_1$ Mice (CFSAN)	Determine the effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F$_1$ mice.
E0212101	**Doerge**	Active/ **Bio Tox/** AGNT	Development of Analytical Methods for Determination of Urethane	1. Develop analytical methods to assess purity and stability of urethane and ethanol that will be used as test compounds in the NTP rodent bioassay; 2. Develop analytical methods to quantify urethane and ethanol content in aqueous dosing solutions and determine stability under storage and use conditions for the NTP bioassay; 3. Develop analytical procedures to quantify the content of urethane in rodent feed.
E0212201 E0212211 E0212213 E0212214 E0212215 E0212221	**Delclos** Ali Ferguson Germolec Newbold Scallet Slikker Weis	Active/ **Bio Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats (Without Behavioral Breeding)	Determine the doses of genistein to be used in a multigeneration bioassay for establishing the effects of this naturally occurring isoflavone on development of reproductive organs, reproduction, cancer of the reproductive organs, and neurological and immunological function.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0212301 E0212311 E0212313 E0212314 E0212315 E0212321 E0212322	**Delclos** Ali Ferguson Germolec Newbold Scallet Slikker Weis	Active/ **Bio Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Methoxychlor Administered Feed to CD (Sprague-Dawley) Rats	Determine the doses of methoxychlor for use in a multigeneration bioassay for assessing the effects of this pesticide on the development of the reproductive tract, reproduction, cancer of the reproductive organs, and neurological and immunological function.
E0212401	**Howard** Bucci Couch Doerge	Active/ **Bio Tox/** AGNT	Comparative Toxicity of Fumonisin Derivatives in Female B6C3F_1 Mice	Compare the toxicity of several fumonisin derivatives in female B6C3F_1 mice.
E0212501 E0212511 E0212513 E0212514 E0212515	**Delclos** Ali Ferguson Germolex Meredith Newbold Scallet Slikker Weis	Active/ **Bio Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Nonylphenol Administered in the Feed to CD (Sprague-Dawley) Rats	Determine the doses of nonylphenol for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.
E0212601 E0212611 E0212613 E0212614 E0212615	**Delclos** Ali Ferguson Germolec Meredith Newbold Scallet Slikker Weis	Active/ **Bio Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Vinclozolin Administered in the Feed to CD (Sprague-Dawley) Rats	Determine the doses of vinclozolin for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.
E0212701	**Culp** Beland Mulligan	Active/ **Bio Tox/** AGNT	Two-Year Bioassay in Mice Administered Malachite Green or Leucomalachite Green in the Diet (CVM)	Determine the risk associated with exposure to malachite green or leucomalachite green.
E0212801	**Culp** Beland Mulligan	Active/ **Bio Tox/** AGNT	Two-year Bioassay in Rats Administered Malachite Green or Leucomalachite Green in the Diet (CVM)	Determine the risk associated with exposure to malachite green or leucomalachite green.
E0212901 E0212911 E0212913 E0212914 E0212915	**Delclos** Ali Ferguson Germolec Meredith Newbold Scallet Slikker Weis	Active/ **Bio Tox/** AGNT	Range Finding Study for the Evaluation of the Effects of Ethinyl Estradiol Administered in the Feed to CD (Sprague-Dawley) Rats During Development	Determine the doses of ethinyl estradiol (EE2) for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0213101 P00402	**Howard** Beer Miller Wamer	Active/ **Bio Tox/** AGNT	The Effects of Chemo-exfoliation using ∀- and ∃-hydroxy Acids on Cell Proliferation and DNA Damage Formation in SKH-1 Mice Exposed to Simulated Solar Light (CDRH)	The NIEHS/FDA Phototoxicity Center is designed to address the effects of compounds on the induction of skin cancer in mice using light sources that are relevant to humans. Input into the design of the facility has been obtained from experts in phototoxicity and photocarcinogenicity. These experts will continue to provide critical advice on the design of the experimental protocols. As a result, a facility will be developed that will meet the rigors of scientific scrutiny, and will generate data for human health risks from the effects of compounds on light-induced skin cancer. The facility is also designed for expansion to allow simultaneous examination of the toxicity or cocarcinogenicity of compounds in the presence of either simulated sunlight or fluorescent UV light. The mechanistic studies in this proposal will provide the data necessary to design and interpret properly the future ∀-hydroxy acid and simulated solar light cocarcinogenicity studies.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0213201 E0213211 E0213213 X80003 X90020	**Delclos** Blaydes Branham Dalu Deorge Ferguson Flynn Laurenzana Newbold Scallet Sheehan Weis	Active/ **Bio Tox/** AGNT	Genistein: Evaluation of Reproductive Effects Over Multiple Generations and the Chronic Effects of Exposure During Various Life Stages	1. Determine the effects of genistein, a naturally occurring isoflavone, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations; 2. Determine if subtle effects observed in the dose range finding study are magnified through multiple generations; 3. Evaluate the reversibility of any observed effects; and 4. Evaluate the chronic toxicity of genistein, particularly potential induction of cancer of the reproductive organs, following exposures that will include various life stages (*in utero* through early adulthood, *in utero* and continuous for two years after birth, *in utero* and lactational only, and postweaning only).

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0213301	**Chou** Beger Chan Doerge Fu Nichols Von Tungeln Yan	In Review/ **Bio Tox/** PRED	A Study of Genotoxic and Secondary Mechanisms of Riddelliine Carcinogenesis	1. Study the mechanisms of direct-acting genotoxicity (involving exogenous DNA adduct formation) of Riddelliine. 2. Determine whether or not secondary mechanisms are involved in Riddelliine-initiated carcinogenesis. 3. Determine whether or not Riddelliine-induced genotoxicity is associated with DNA cross-linking. 4. Analyze Riddelliine-derived exogenous and endogenous DNA adducts in target tissues from rats treated with Riddelliine under the NTP chronic study and from male and female rats treated for a shorter period of time with Riddelliine and its reactive metabolite, dehydroRiddelliine. 5. Analyze and compare the hepatic DNA adducts formed in Riddelliine-treated rats, and in rats treated with two other tumorigenic pyrrolizidine alkaloids, retrorsine and monocrotaline, as well as their reactive metabolites, dehydroretrorsine and dehydromonocrotaline. 7. Compare the metabolic activation pathways and DNA adduct formation of Riddelliine, retrorsine, and monocrotaline between rat and human liver microsomal systems.
E0213401	**Laurenzana** Blaydes Delclos	In Review/ **Bio Tox/** AGNT	Effect of Endocrine Active Compounds on Steroid Hormone Metabolism in Receptor Expression in Human Hepatoma, Breast, and Prostate Cancer Cell Lines	Determine potential mechanisms through which the endocrine active compounds methoxychlor, genistein, vinclozolin, nonylphenol, and 17∀-ethinyl estradiol may affect endocrine function. The data obtained from these studies will be useful in determining if these have similar mechanisms of action in rats and humans.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
P00392	**Doerge** Holder Sittonen	Active/ **Bio Tox/** METH	Development of Analytical Methods for Ethinylestradiol in Rodent Feed	The hypothesis that environmental chemicals with estrogenic activity cause reproductive problems and cancer of the reproductive tract in humans is based in part on the adverse outcomes observed in wildlife and the known effects of diethylstilbestrol in humans. The potential for reproductive and developmental toxicity of environmental chemicals are the focus of the Endocrine Disruptor Study of the National Toxicology Program in conjunction with NCTR. As part of this study, the oral contraceptive agent ethinylestradiol will be tested by lifelong feeding to rats and following the animals through multiple generations for adverse effects, including carcinogenesis. Central to these studies is the ability to quantify the content of ethinylestradiol in dosing medium. Because of the high potency of ethinylestradiol, the challenge of this project will be coupling a low dosing level with the complex suite of coextractive compounds found in rodent feed.
X60072	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies (F0 Generation for Reproductive Assessment–Methoxychlor	Project under development.
X60074	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F0 Generation–Cancer/Breeding – Methoxychlor	Project under development.
X60077	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F1 Generations–Reproduction – Methoxychlor	Project under development.
X60078	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies – F2 –Reproduction–Methoxychlor	Project under development.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
X60079	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F3 Generations –Reproduction – Methoxychlor	Project under development.
X60080	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F3 Generation-Cancer– Methoxychlor	Project under development.
X60081	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F4 Generations – Reproduction – Methoxychlor	Project under development.
X60092	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies F0 Generation for Reproductive Assessment – Nonylphenol	Project under development.
X60097	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F1 Generations – Reproduction – Nonylphenol	Project under development.
X60098	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F2 –Reproduction – Nonylphenol	Project under development.
X60099	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F3 Generations – Reproduction – Nonylphenol	Project under development.
X60101	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F4 Generations – Reproduction – Nonylphenol	Project under development.
X60112	**Delclos** Weis	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies- F0 Generations – Reproductive Vinclozolin	Project under development.
X60117	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F1 Generations – Reproduction – Vinclozolin	Project under development.
X60118	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F2 – Reproduction – Vinclozolin	Project under development.
X60119	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F3 Generations – Reproduction – Vinclozolin	Project under development.
X60121	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F4 Generations – Reproduction – Vinclozolin	Project under development.
X60122	**Delclos** Weis	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies F0 Generation for Reproductive Assessment-Ethinyl Estradiol	Project under development.
X60127	**Delclos** Weis	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F1 Generations –Reproduction – Ethinyl Estradiol	Project under development.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
X60128	**Delclos** Weis	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F2 Generations – Reproduction – Ethinyl Estradiol	Project under development.
X60129	**Delclos** Weis	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F3 Generations – Reproduction – Ethinyl Estradiol	Project under development.
X60131	**Delclos** Weis	Proposed/ **Bio Tox/** AGNT	Multigeneration Studies - F4 Generations – Reproduction – Ethinyl Estradiol	Project under development.
X90008	**Delclos** Miller	Proposed/ **Bio Tox/** AGNT	New Method for Analysis of Vinclozolin	Project under development.

Cooperating Organization: Scientific Instrument Services, Inc.

Project Number	**Principal**/ Co-Principal Investigator(s)	Status/ **Res. Area**/ Goal	Title	Objective
E0697201	**Wilkes** Abramson Billedeau Freeman Heinze Pothuluri	Active/ **Chem/** METH	Universal Interface Development and Applications	Develop, if possible and practical, a variety of new technologies for improving high performance liquid chromatography (HPLC) detection. By eliminating hazards associated with radioactivity, it can make possible metabolic drug studies involving human subjects. Several CRADAs will be negotiated during the work to facilitate development of commercial versions of the devices, which show the most promise.

PUBLICATIONS

1998 NCTR PUBLICATIONS

The following list of NCTR publications includes those publications which were accepted for publication or published during the fiscal year, 1998.

Proceeding each publication (in parenthesis) is:

1. The NCTR project number associated with the publication, if any;

   [Ex. **(E0XXXXXX)**]

2. The strategic research goal (defined in the "Preface" of this book)

   [Ex. **(CONCEPT-DRIVEN)**]

3. The primary division responsible for the publication and the collaborating division(s). The primary and collaborating divisions represent the current division of the author or coauthor at the time of the publication of this book and are abbreviated as shown below:

   [Ex. **(Bio Tox)** (Neuro Tox) (Biometry)]

Division/Contractor Abbreviations:

Biochemical Toxicology (Bio Tox)
Biometry & Risk Assessment (Biometry)
Chemistry (Chem)
Division of Facilities, Engineering Management (DFEM)
Genetic & Reproductive Toxicology (Gen & Repro)
Microbiology (Micro)
Molecular Epidemiology (Mol Epi)
Neurotoxicology (Neuro Tox)
Office of Director, Immediate Office (OD/Imm Off)
Office of Research, Immediate Office (OR/Imm Off)
Office of Management, Facilities & Research Support, Immediate Office (OMFRS/IO)
Pathology Associates, Inc. (Pathology)
Technology Advancement (Tech Adv)
Veterinary Services (Vet Svcs)

1998 NCTR Publications

1. Ahn, H. and Kodell, R.L. Efficient designs for animal carcinogenicity experiments. Communications in Statistics - Theory and Methods, 27(6):1275-1287. Accepted: 10/1/97**. (E0689601) (METHOD-DRIVEN) (Biometry)**

2. Ahn, H., Kodell, R.L. and Moon, H. Attribution of tumor lethality in the absence of cause-of-death information. 1998 Proceedings of the Biometric Section, American Statistical Association. Accepted: 8/9/98. **(E0689601) (METHOD-DRIVEN) (Biometry)**

3. Allen, L.B., Siitonen, P.H. and Thompson, H.C. Simultaneous determination of lead and nickel in edible oils with ICP-AES and GFAAS. Journal of American Oil Chemist's Society, 75(4):477-481. Accepted: 10/14/97. **(E0689201) (METHOD-DRIVEN) (Chem)** (Micro)

4. Ambrosone, C.B. and Kadlubar, F.F. Acetylation-related susceptibilities of metabolism. Biomarkers: Medical and Workplace Applications, 189-210. Accepted: 1/10/98. **(METHOD-DRIVEN) (Mol Epi)**

5. Ambrosone, C.B. and Thompson-Carino, P. Molecular Epidemiology of Epithelial Tumors. Current Opinions in Oncology, 10:467-474. Accepted: 12/15/97. **(PREDICTION OF TOXICITY) (Mol Epi)**

6. Ambrosone, C.B., Coles, B.F., Freudenheim, J.L. and Shields, P.G. Glutathione S-transferase (GST M1) genetic polymorphisms, dietary antioxidants and risk of breast cancer. American Journal of Nutrition (SUPL), In Press. Accepted: 9/25/98. **(PREDICTION OF TOXICITY) (Mol Epi)**

7. Ang, C.Y. Health implications of Asian diets and supplements. In: Asian Foods: Science and Technology, Chapter 16. Accepted: 6/25/98. **(E0702501) (AGENT-DRIVEN) (Chem)**

8. Arani, R.B. and Chen, J.J. A power study of a sequential method of p-value adjustment for correlated continuous endpoints. Journal of Biopharmaceutical Statistics. Accepted: 6/26/98. **(E0700901) (METHOD-DRIVEN) (Biometry)**

9. Bailey, B., Morris, P.D., McMartin, K., Klein, J., Duhart, H.M., Gillam, M.P., Binienda, Z.K., Slikker, W., Paule, M.G. and Koren, G. Transplacental pharmacokinetics of cocaine and benzoylecgonine in plasma and hair of rhesus monkeys. Reproductive Toxicology, 12(5):517-523. Accepted: 6/20/98. **(E0663300) (AGENT-DRIVEN) (Neuro Tox)**

10. Beland, F.A., Schmitt, T., Fullerton, N.F. and Young, J.F. Metabolism of chloral hydrate in mice and rats after single and multiple doses. Journal of Toxicology and Environmental Health, Part A, 54:101-118. Accepted: 11/2/97. **(E0210101, E0210201, E0210401) (AGENT-DRIVEN) (Bio Tox)** (Micro) (Biometry)

11. Benson, K.A., Ali, S.F. and Wilson, M.C. The effects of prenatal cocaine exposure on dopaminergic challenge and receptor binding in wistar rats. Annals of the New York Academy Sciences, 801:289-300. Accepted: 10/1/97. **(AGENT-DRIVEN) (Neuro Tox)**

12. Bezalel, L., Hadar, Y. and Cerniglia, C.E. Degradation of polycyclic aromatic hydrocarbons by the white-rot fungus *Pleurotus ostreatus*. In: Advances in Biotechnology, pps. 405-421. Accepted: 10/1/98. **(PREDICTION OF TOXICITY) (Micro)**

13. Binienda, Z.K. and Scallet, A.C. Neuroprotective effect of perinatal hypoxia against 3-NPA neurotoxicity. Mitochondrial inhibitors as a tool for neurobiology, Borlogan, Nishino, Sanberg, Humana, Eds., New York, NY. Accepted: 6/29/98. **(E0692301) (AGENT-DRIVEN) (Neuro Tox)**

14. Binienda, Z.K., Beaudoin, M.A., Thorn, B.T., Prapurna, R.D., Johnson, J.R., Fogle, C.M., Slikker, W. and Ali, S.F. Alterations in electroencephalogram and monoamine concentrations in rat brain following ibogaine treatment. Annals of the New York Academy of Sciences, 844:265-273. Accepted: 1/25/98. **(E0698301) (AGENT-DRIVEN) (Neuro Tox)** (ROW)

15. Binienda, Z.K., Simmons, C.E., Hussain, S.M., Slikker, W. and Ali, S.F. Effect of acute exposure to 3-nitropropionic acid on activities of endogenous antioxidants in the rat brain. Neuroscience Letters, 251:173-176. Accepted: 6/19/98. **(E0701001) (CONCEPT-DRIVEN) (Neuro Tox)** (Pathology)

16. Bishop, M.E., Aidoo, A., Domon, O.E., Morris, S.M. and Casciano, D.A. Phenolphthalein induces micronuclei in transgenic human lymphoblastoid cells. Environmental and Molecular Mutagenesis - Brief Communication, 32:286-288. Accepted: 7/23/98. **(E0695001) (PREDICTION OF TOXICITY) (Gen & Repro Tox)**

17. Bowyer, J.F., Frame, L.T., Nagamoto-Combs, K., Clausing, P.P., Tank, A., Osterhout, C.A. and Sterling, C.R. Long-term effects of amphetamine neurotoxicity on tyrosine hydroxylase mRNA and protein in aged rats. Journal of Pharmacology and Experimental Therapeutics, 286:1074-1085. Accepted: 4/29/98. **(AGENT-DRIVEN) (Neuro Tox)**

18. Bowyer, J.F., Peterson, S.L., Rountree, R.L., Tor-Agbidye, J. and Wang, G.J. Neuronal degeneration in rat forebrain resulting from d-amphetamine-induced convulsions is dependent on seizure severity and age. Brain Research. Accepted: 8/10/98. **(E0702401) (CONCEPT-DRIVEN) (Neuro Tox)**

19. Broening, H.W. and Slikker, W. Ontogeny of neurotransmitters: monoamines. Handbook of Developmental Neurotoxicology, Slikker and Chang, Eds., Academic Press, San Diego. Accepted: 5/1/98. **(PREDICTION OF TOXICITY) (Neuro Tox)**

20. Castlebury, L.A., Sutherland, J.B., Tanner, L.A., Henderson, A. and Cerniglia, C.E. Use of a bioassay to evaluate the toxicity of beauvericin to bacteria. World J. Microbiol. Biotechnol., Accepted: 9/11/98. **(E0692201) (AGENT-DRIVEN) (Micro)** (EHPAS)

21. Cerniglia, C.E. Assessing the effects of antimicrobial residues in food on the human intestinal microflora. WHO Technical Report Series 876, Geneva, pp. 71-85. Accepted: 11/14/97. **(PREDICTION OF TOXICITY) (Micro)**

22. Cerniglia, C.E. Current testing approaches for the assessment of the effects of veterinary drug residues in food on the human intestinal microflora. IBC UK Conference on Global Developments in Risk Assessments of Residues of Veterinary Drugs in Brussels, 1-15. Accepted: 9/1/98. **(METHOD-DRIVEN) (Micro)**

23. Cerniglia, C.E. Valuation of the safety of veterinary drug residues in food and effects on the human intestinal microflora. 35th Meeting of the Brazilian Society of Animal Science, 255-268. Accepted: 7/27/98. **(PREDICTION OF TOXICITY) (Micro)**

24. Chelonis, J.J. and Logue, A.W. Effects of reinforcer type on rats' sensitivity to variation in reinforcer amount and reinforcer delay. Behavioural Processes, 39:187-203. Accepted: 10/15/97. **(CONCEPT-DRIVEN) (Neuro Tox)**

25. Chelonis, J.J., Logue, A.W., Sheehy, R. and Mao, J. Effects of response effort on self-control in rats. Animal Learning and Behavior. Accepted: 7/15/98. **(CONCEPT-DRIVEN) (Neuro Tox)**

26. Chen, J.B., Dass, S.B., Burkhart, J.G. and Heflich, R.H. Sensitivity of the PhiX174 am3 allele in relation to the endogenous *hprt* gene for detecting mutation in transgenic mice. Environmental and Molecular Mutagenesis, 32:229-235. Accepted: 8/9/98. **(E0697701) (PREDICTION OF TOXICITY) (Gen & Repro Tox)**

27. Chen, J.J. Analysis of reproductive and developmental studies. In: Design and analysis of animal studies in Pharmaceutical., Marcel Dekker, Inc., New York, NY, Eds., Shein-Chung Chow and Jen-Pei Liu, pp. 309-355. Accepted: 6/1/97. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

28. Chen, J.J. P-value adjustment for multiple binary endpoints. Communications in Statistics - Theory and Methods, 27(11):2791-2806. Accepted: 6/23/98. **(E0700901) (METHOD-DRIVEN) (Biometry)**

29. Chen, J.J. and Ahn, H. Marginal models with multiplicative variance components for over-dispersed binomial data. Journal of Agricultural, Biological and Environmental Statistics, 2(4):440-450. Accepted: 10/2/97. **(E0684300) (METHOD-DRIVEN) (Biometry)**

30. Chen, R., Chou, M.W. and Ueng, T. Induction of cytochrome P450 1A in hamster liver and lung by 6-nitrochrysene. Archives in Toxicology. Accepted: 8/1/98. **(AGENT-DRIVEN) (Bio Tox)**

31. Chen, T., Aidoo, A., Manjanatha, M., Mittelstaedt, R.A., Shelton, S.D., Lyn-Cook, L.E., Casciano, D.A. and Heflich, R.H. Comparison of mutant frequencies and types of mutations induced by thiotepa in the endogenous *hprt* gene and transgenic *laci* gene of Big Blue(R) rats. Mutation Research, 403:199-214. Accepted: 4/8/98**. (E0695801) (PREDICTION OF TOXICITY) (Gen & Repro Tox)**

32. Chung, K., Lu, Z. and Chou, M.W. Effects of tannic acid and related compounds on growth of some intestinal bacteria. Food and Chem Tox. Accepted: 8/1/98. **(E0260401) (CONCEPT-DRIVEN) (Bio Tox)**

33. Clausing, P.P., Newport, G.D. and Bowyer, J.F. Fenfluramine levels in brain microdialysate, brain tissue and plasma of rats administered doses of d-fenfluramine known to deplete 5-hydroxytryptamine levels in brain. Journal of Pharmacology and Experimental Therapeutics, 284:618-624. Accepted: 10/7/97. **(E0690301) (CONCEPT-DRIVEN) (Neuro Tox)**

34. Collins, T.F., Shackleford, M.E., Sprando, R., Laborde, J.B., Hansen, D.K., Eppley, R., Trucksess, M. and Howard, P. Effects of fumonisin B_1 in pregnant rats. Food and Chemical Toxicology, 36:397-408. Accepted: 6/1/98. **(E0211001) (AGENT-DRIVEN) (Gen & Repro Tox)** (Bio Tox)

35. Collins, T.F., Sprando, R., Hansen, D.K. and Shackleford, M.E. Testing guidelines for evaluation of reproductive and developmental toxicity of food additives in females. International Journal of Toxicology, 17:299-325. Accepted: 3/1/98. **(METHOD-DRIVEN) (Gen & Repro Tox)**

36. Collins, T.F., Sprando, R., Shackleford, M.E., Laborde, J.B., Hansen, D.K., Eppley, R., Trucksess, M. and Howard, P. Effects of fumonisin B_1 in pregnant rats. Part 2. Food and Chemical Toxicology, 36:673-685. Accepted: 7/1/98. **(AGENT-DRIVEN) (Gen & Repro Tox)**

37. Cooney, C.A., Wise, C.K., Poirier, L.A. and Ali, S.F. Methamphetamine treatment affects blood and liver s-adenosylmethionine (SAM) in mice: Correlation with dopamine depletion in the striatum. Annals N.Y. Academy of Sciences, 844:191-200. Accepted: 3/1/98. **(E0683000) (CONCEPT-DRIVEN) (Mol Epi)** (Neuro Tox)

38. Darsey, J.A., Lay, J.O. and Holland, R.D. Deconvolution of composite mass spectra by artificial neural networks. International Journal of Biotechnology. Accepted: 8/15/98. **(E0700501) (METHOD-DDRIVEN) (Chem)**

39. Dass, S.B., Hammons, G.J., Bucci, T.J., Heflich, R.H. and Casciano, D.A. Susceptibility of C57BL/6 mice to tumorigenicity induced by dimethylnitrosamine and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in the neonatal bioassay. Cancer Letters, 124:105-110. Accepted: 10/13/97. **(E0693301) (PREDICTION OF TOXICITY) (Gen & Repro Tox)** (Mol Epi) (Pathology)

40. Dass, S.B., Heflich, R.H. and Casciano, D.A. Mutational response at the splenic T-lymphocyte *hprt* locus in mice treated as neonates with N-ethyl-N-nitrosourea, dimethylnitrosamine and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Environmental and Molecular Mutagenesis, 31:243-247. Accepted: 11/11/97. **(E0693301) (PREDICTION OF TOXICITY) (Gen & Repro Tox)**

41. Doerge, D.R., Holder, C.L., Major, H., Churchwell, M.I. and Giles, R. Analysis of conjugated soy isoflavones in soy products and human blood using LC-MS/MS and LC/MS. Biochemical Pharmacology. Accepted: 10/1/98. **(AGENT-DRIVEN) (Chem)** (Micro)

42. Fairweather, J., Kodell, R.L. and Lin, K.K. Biostatistical methodology in carcinogenicity studies. Drug Information Journal, 32:401-421. Accepted: 11/4/97**. (P00268) (CENTER SUPPORT RESEARCH) (Biometry)**

43. Ferguson, S.A. and Holson, R.R. Neonatal dexamethasone on day 7 causes mild hyperactivity and cerebellar stunting. Neurotoxicology and Teratology. Accepted: 6/19/98**. (E0683000) (CONCEPT-DRIVEN) (Neuro Tox)**

44. Ferguson, S.A., Holson, R.R., Gazzara, R.A. and Siitonen, P.H. Minimal behavioral effects from moderate postnatal lead treatment. Neurotoxicology and Teratology. Accepted: 5/26/98. **(E0690401) (PREDICTION OF TOXICITY) (Neuro Tox)** (Gen & Repro Tox) (Chem)

45. Frederick, D.L., Ali, S.F., Gillam, M.P., Gossett, J., Paule, M.G. and Slikker, W. Acute effects of dexfenfluarmine (d-FEN) and methylenedioxymethamphetamine (MDMA) before and after short-course, high-dose treatment. Annals of the New York Academy of Sciences, 844:183-190. Accepted: 5/30/98. **(AGENT-DRIVEN) (Neuro Tox)** (ROW)

46. Fu, P.P., Von Tungeln, L.S., Chiu, L., Zhan, D., Deck, J. and Bucci, T.J. Structure, tumorigenicity, microsomal metabolism, and DNA binding of 7-Nitrodibenz[a,h]-anthracene. Chem. Res. Toxicol., 11:937-945. Accepted: 6/25/98**. (E0657300) (PREDICTION OF TOXICITY) (Bio Tox)** (Micro) (Pathology)

47. Fu, P.P., Von Tungeln, L.S., Xia, Q., Yi, P., Casciano, D.A., Flammang, T.J. and Kadlubar, F.F. Neonatal mouse tumorigenicity bioassay. The Drug Information Journal, 32:711-728. Accepted: 12/1/97. **(E0687901) (PREDICTION OF TOXICITY) (Bio Tox)**

48. Gaylor, D.W. Safety assessment with hormetic effects. Human and Experimental Toxicology, 17:251-253. Accepted: 1/5/98. **(S00116) (CONCEPT-DRIVEN) (OD/Imm Off)**

49. Gaylor, D.W. and Gold, L.S. Regulatory cancer risk assessment based on a quick estimate of a benchmark dose derived from the maximum tolerated dose. Regulatory Toxicol. and Pharmacol. Accepted: 9/22/98**. (S00116) (CONCEPT-DRIVEN) (OD/Imm Off)**

50. Gaylor, D.W., Kodell, R.L. and Chen, J.J. A unified approach to risk characterization. Inhalation Toxicology. Accepted: 4/28/98. **(PREDICTION OF TOXICITY) (OD/Imm Off)** (Biometry)

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53. Goodwin, T.E., Holland, R.D., Lay, J.O. and Raney, K.D. A simple, manual procedure for solid phase synthesis of peptide nucleic acids (PNAs) with N-Terminal cysteine. Bioorganic and Medicinal Chem. Letters. Accepted: 7/28/98. **(METHOD-DRIVEN) (Chem)**

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55. Gu, Y., Branham, W.S., Sheehan, D.M. and Streck, R.D. Expression of insulin-like growth factors, insulin-like growth factor receptors, and insulin-like frowth factor binding proteins in the rat uterus. '97 International Symposium for Young Chinese Reproductive Biologists, 64-70. Accepted: 10/29/97. **(CONCEPT-DRIVEN) (Gen & Repro Tox)**

56. Hammons, G.J., Fletcher, J.V., Stepps, K.R. and Smith, E.M. Effects of chemoprotective agents on the metabolic activation of the carcinogenic arylamines PhiP and 4-ABP in human and rat liver microsomes. Nutrition and Cancer, In Press. Accepted: 9/15/98. **(E0689401) (PREDICTION OF TOXICITY) (Mol Epi)**

57. Hansen, D.K. Developmental neurotoxicity of antiepileptic drugs. Academic Press, 643-660. Accepted: 1/14/98**. (AGENT-DRIVEN) (Gen & Repro Tox)**

58. Harris, A.J., Shaddock, J.G., Manjanatha, M., Lisenbey, J.A. and Casciano, D.A. Identification of differentially expressed genes in aflatoxin B_1-treated Fischer 344 rats and cultured primary rat hepatocytes. Carcinogenesis, 19(8):1451-1458. Accepted: 4/28/98. **(E0690601) (PREDICTION OF TOXICITY) (Gen & Repro Tox)**

59. Hashemi, R.R., Slikker, W., Terry, M.S., Paule, M.G. and Tyler, A.A. Analysis of reducts in diagnostic systems. Proceedings of the 1998 World Automation Congress. Accepted: 5/1/98**. (METHOD-DRIVEN) (Neuro Tox)**

60. Hashemi, R.R., Terry, M.S., Tyler, A.A., Slikker, W. and Paule, M.G. The development of profiles for children with attention deficit disorder and monkeys: a neural network approach. Proceedings for 1998 ACM Symposium on Applied Computing (SAC '98), 50-54. Accepted: 11/27/97. **(PREDICTION OF TOXICITY) (Neuro Tox)**

61. Hussain, S.M., Hass, B.S., Slikker, W. and Ali, S.F. Reduced levels of catalase activity potentiate MPP+ induced toxicity: Comparison between MN9D cells and CHO cells. Toxicology Letters. Accepted: 9/2/98. **(E0671900) (AGENT-DRIVEN) (Neuro Tox)** (Gen & Repro Tox)

62. Itzhak, Y., Martin, J.L., Black, M.D. and Ali, S.F. Effect of melatonin on methamphetamine-and1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurotoxicity and methamphet-amine-induced behavioral sensitization. Neuropharmacology, 37:781-791. Accepted: 11/15/97. **(AGENT-DRIVEN) (Neuro Tox)**

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