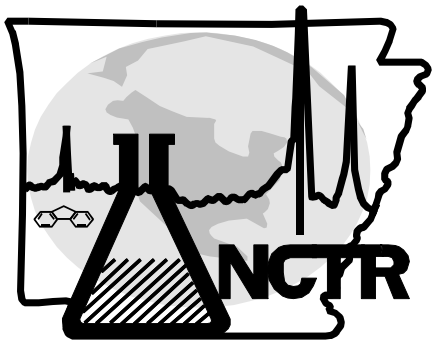
NCTR
Research Accomplishments and Plans

FY 1997-1998



Leaders in Health Science Research for FDA

Jefferson Laboratories of the FDA

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PREFACE

The National Center for Toxicological Research (NCTR) is a research facility of the Jefferson Laboratories of the Food and Drug Administration (FDA). The Jefferson Laboratories of the FDA is comprised of both NCTR and the Arkansas Regional Laboratory (ARL) of the Office of Regulatory Affairs (ORA), and is located near Jefferson, a rural community in south-central Arkansas approximately 30 miles from Little Rock. 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 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 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 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 six research divisions whose goals, ongoing research accomplishments and FY 98 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.

FY 97 Accomplishments

As part of its ongoing review of the scientific programs at the NCTR, members of the SAB along with expert consultants, conducted two site visits to evaluate the Center's pilot project on the establishment of the Estrogen Knowledge Base (EKB); and, for the first time, evaluated a research support function, the Center's Information Management (IM) Program. Draft reports from both site visits were presented to the full SAB at the June 5-6, 1997, meeting.

The Board accepted the site-visit team's (SVT) draft report on the EKB project and its recommendations as written. Copies of that report are available from the Executive Secretary.

The Board also discussed the draft report from the IM SVT. The SVT Chair recommended that no action be taken on this report to allow two members of the team the opportunity to file a minority report. Final action on the draft report was postponed until the next full meeting of the SAB which is scheduled to be held May 6-7, 1998.

A progress report was provided to the Board on the implementation of their recommendations on the Center's Analytical Methods Development Program. The members expressed their satisfaction with the Center's leadership in utilizing the guidance the Board provided in the first round of the site-visit reviews.

A schedule for the next round of program evaluations was established for FY 98 that includes

review of the Biometry and Risk Assessment, Neurotoxicology, and Carcinogenesis programs.

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	6/30/98	Veterinary Medicine, Biochem./Pharm.
Dr. Robert E. Anderson Professor Emeritus, West Virginia University	WV School of Environmental Education, Inc. Morgantown, WV	6/30/00	Food Technology
Dr. William R. Bruce Professor, Departments of Medical Biophysics and Nutritional Science	University of Toronto Don Mills, Ontario	6/30/99	Medicine, Biophysics
Dr. Harold Davis Director of Toxicology	AMGEN (Applied Molecular Generics) Thousand Oaks, CA	6/30/98	Pathology, Veterinary Medicine
Dr. Tómas R. Guilarte Professor, School of Hygiene and Public Health	Johns Hopkins University Baltimore, MD	6/30/99	Medical Physics, Zoology
Dr. Joseph V. Rodricks Senior Vice President	ENVIRON International Corporation Arlington, VA	6/30/99	Toxicology/Risk Assessment
Dr. Marcy E. Rosenkrantz Associate Director	Cornell Theory Center, Cornell University Ithaca, 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
Dr. Lily Y. Young Professor of Microbiology AgBiotech & Department of Environmental Sciences	Rutgers University Cook College New Brunswick, NJ	6/30/98	Microbiology
Mr. Ronald F. Coene Executive Secretary Deputy Director, Washington Operations, NCTR	FDA/NCTR Rockville, MD	Ongoing	Research Administration

*Committee Chair

RESEARCH ACCOMPLISHMENTS AND PLANS

BIOCHEMICAL TOXICOLOGY

BIOCHEMICAL TOXICOLOGY

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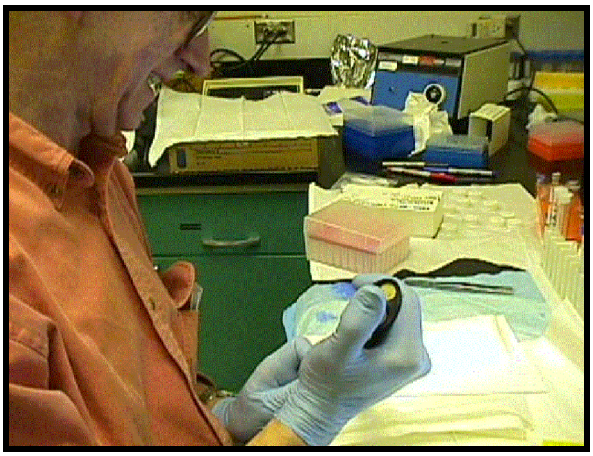

Assaying for ceramide synthase as part of the NTP fumonisin study.

Introduction

This year one out of every five deaths within the United States will be due to cancer. Since it has been estimated that 60-90% of these cancers result from exposure to environmental or exogenous agents, a significant proportion of this disease should be preventable through controlling exposure to these factors. Cigarette smoking, which accounts for nearly 30% of all cancer deaths, is clearly the major identifiable cause of cancer in our society. The agents responsible for the remainder are more varied and are believed to be due primarily to trace substances found in food, water, air, medicines, and cosmetics. A number of substances carcinogenic to animals, including mycotoxins, nitrosamines, urethanes, heterocyclic aromatic amines, and hydrazines, have been identified in foods and beverages. Likewise, in recent years, certain widely used drugs, such as methapyrilene, methylphenidate, 3'-azido-3'-deoxythymidine (AZT), and tamoxifen have been demonstrated to be carcinogenic in animals. Considering the amounts of food, beverages, and drugs that are consumed, along with the carcinogenic potency of substances contained within these groups, it appears that these three sources are potential, major contributors to the incidence of human cancers.

FY 98 Goals

In the area of biochemical toxicology, the major goals for FY 98 are to assist other FDA centers in their regulatory mandate by: 1) conducting chronic bioassays and associated mechanistic studies to assess the carcinogenic risk for specific chemicals and substances, including secondary or indirect-acting carcinogens; and 2) introducing new techniques to assess carcinogenic risk.

FY 97 Accomplishments

In response to requests made to the National Toxicology Program (NTP) by the Center for Drug Evaluation and Research (CDER) and the Center for Food Safety and Applied Nutrition (CFSAN), chronic bioassays were conducted during FY 97 on the pediatric sedative chloral hydrate and the mycotoxin fumonisin B_1 (FB_1). The results of these bioassays will be used by CDER and CFSAN to establish the risks associated with exposure to these compounds, which will form the basis for regulatory decisions. Likewise, bioassays on nitropolycyclic aromatic hydrocarbons, air particulate samples, coal tar, and benzo[*a*]pyrene have been conducted to assist other agencies in their regulatory mandate. As an example, a risk assessment was performed using the tumorigenicity data acquired with benzo[*a*]pyrene and coal tar. At the request of the Center for Veterinary Medicine (CVM), subchronic bioassays and mechanistic studies were initiated on malachite green, a therapeutic agent used in aquaculture. The results from these experiments have been used to design a chronic bioassay that will begin during FY 98. In response to a request from CFSAN, a chronic bioassay and associated mechanistic studies were started to elucidate the effects of ethanol upon the carcinogenicity of urethane. The data from these studies will be used to assess the risk from the exposure to urethane in alcoholic beverages.

Toxicities associated with endocrine disrupting chemicals have recently become a major public health issue. In response to this concern, a major new initiative was funded by the NTP. This program, which will focus on reproductive and carcinogenic endpoints, will investigate a variety of chemicals including genistein, methoxychlor, nonylphenol, vinclozolin, and ethinyl estradiol. A major emphasis of this program will be to elucidate dose-response relationships over a wide range of doses. During the current fiscal year, range-finding and immunotoxicity studies were initiated on genistein, methoxychlor, and nonylphenol.

Traditional chronic carcinogenicity bioassays are both very expensive and lengthy; thus, the development of alternative methods of assessing carcinogenic potential would 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. Currently, in collaboration with investigators at CDER, this alternative bioassay has been applied to benzodiazipenes (oxazepam, diazepam, chlordiazepoxide, midazolam, and flurazepam), antihistamines (methapyrilene, doxylamine, pyrilamine, chlorpheniramine, diphenhydramine, promethazine, terfenedine, and hydroxyzine), lipid peroxidation products (4-hydroxy-2-nonenal, malondialdehyde, crotonaldehyde, and acrolein), and other miscellaneous chemicals (phenolphthalein, tacrine, methylphenidate, aflatoxin B_1, 6-nitrochrysene, 4-aminobiphenyl, and benzo[*a*]pyrene). Compounds currently being investigated in the neonatal mouse tumorigenicity assay include estrogens and antiestrogens

(β-estradiol, 2-hydroxyestradiol, 4-hydroxyestradiol, 16-hydroxyestradiol, diethylstilbestrol, tamoxifen, toremifene, genistein, daidzein, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), and benzene hexachloride) and peroxisome proliferators and lipid peroxidation inducers (chloral hydrate, carbon tetrachloride, methylene chloride, chlordane, heptachlor, paraquat, tifluralin, di(2-ethylhexyl)phthalate, and chlorbrate).

The covalent interaction of carcinogens with DNA is a common critical event in carcinogenesis. If the structure of the adduct is known, the appropriate immunogen can be synthesized and antisera can be raised. Within the biochemical toxicology area, an ongoing goal is to exploit both the immunogenicity and the antigenicity of these adducts to develop and apply immunochemical methods to address problems of regulatory concern including exposure, risk of toxicity, product screening, and mechanisms of toxicity. During the current year, this technology was extended to fumonisin B_1, fumonisin B_2, and fumonisin B_3, and the etheno-type DNA adducts formed by urethane. A major focus of this work was the preparation and use of selective immunoaffinity matrices to isolate and concentrate low abundance analytes from complex biological mixtures.

Recently, much public attention has been given to the controversy over the safety of silicone breast implants. An even larger issue is a general dearth of knowledge concerning the safety of biocompatible materials. A goal of the biochemical toxicology group is to establish a strong research effort in this relatively neglected area. This research will increase knowledge of the mechanisms of long-term toxicity of implanted materials and thus increase the scientific validity of regulatory decisions pertaining to the safety of materials intended for prolonged residence in the body. During the current year, immunohistochemical techniques were used to examine acute inflammatory and chronic fibrotic responses at the biomaterial-cellular interface. This study, which was funded by a grant from the FDA's Office of Women's Health, demonstrated that mutations in the p53 tumor suppressor gene were induced during foreign body carcinogenesis. In additional work, a transgenic mouse experiment was initiated to test the hypothesis that biomaterials can act as tumor promoters. Other studies have focused on evaluating sex differences in the responses to biomaterials, strain differences in the extent of oxidative damage induced by foreign bodies, and serum albumin adducts as biomarkers for exposure to toluenediamines, carcinogenic aromatic amines that have been observed in women with polyurethane-covered breast implants.

In order to help interpret the carcinogenicity bioassays being conducted with fumonisin B_1, a number of additional studies were conducted, including investigating the mechanism of action of fumonisin B_1, understanding the pharmacokinetics of fumonisin B_1 in rodents and nonhuman primates, identifying the genotoxicants in *Fusarium*, and monitoring experimental animals and exposed human populations for *Fusarium*-derived DNA adducts. These studies indicate that the toxicities of fumonisin B_1 may be elicited through the induction of programmed cell death (apoptosis), which can act as a promoting stimulus for cells initiated

by genotoxic chemicals produced by *Fusarium*. Similar mechanistic studies have been conducted with chloral hydrate, malachite green, polycyclic aromatic hydrocarbons, nitropolycyclic aromatic hydrocarbons, aromatic amines, and urethane.

A major new focus within the division has been in the area of dietary folate and methyl deficiency. Recent clinical and experimental data have linked nutritional folic acid status to both anticarcinogenic and procarcinogenic activities. Using an *in vitro* model, investigators within the division have demonstrated that folate supplementation of normal cells has a protective effect, but that once cells are irreversibly transformed, excess folate can exacerbate neoplastic progression. Additional studies have focused on the relationships amongst polymorphisms in the methylene tetrahydrofolate reductase gene, folate status, and the incidence of Down Syndrome. Further work demonstrated that feeding methyl-supplemented diets to pregnant mice altered the epigenetically determined coat color pattern and associated phenotype of their offspring.

Although most tumors arise from the covalent interaction of chemical carcinogens with DNA, the correlation between the concentration of DNA adducts and the resultant tumorigenic response is by no means certain. A continuing emphasis within the division has been to elucidate this relationship using tumor models in which potential human carcinogens are administered over wide dose ranges. Current studies have focused on DNA adducts from coal tar, benzo[*a*]pyrene, 2-acetylaminofluorene, tamoxifen, and aflatoxin B_1. Additional endpoints that have been examined include the induction of DNA replication, cell proliferation, apoptosis, and mutations. Other experiments have investigated the relationships between DNA adduct structure and mutagenesis. These data will allow greater confidence in risk estimates based upon DNA adduct determinations.

FY 98 Plans

Method-/Agent-Driven Research

During 1998, 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 continue, and a chronic bioassay with malachite green will be initiated. Range-finding and immunotoxicity studies will begin on the endocrine disrupting chemicals vinclozolin and ethinyl estradiol, while a multigenerational bioassay will be started with genistein. In response to a request from CFSAN, protocols will be prepared to conduct a comprehensive toxicological assessment on α-hydroxy acids, chemoexfoliants that are components of many skin care products.

Mechanistic studies will also continue on fumonisin B_1, malachite green, and urethane in the presence of alcohol. The fumonisin B_1 experiments will be centered on the role of apoptosis

in the tumorigenic response and upon the formation of DNA adducts by other mycotoxins produced by *Fusarium*. Particular emphasis will be placed on the isolation and characterization of ceramide synthase, a key enzyme involved in the toxicities of fumonisin B_1. An extensive short-term study will be conducted 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. Additional mechanistic studies will examine the DNA adducts formed by the mycotoxin riddelliine, a compound of interest to CFSAN that is undergoing a NTP-sponsored chronic bioassay. Mechanistic studies will also be conducted on endocrine disrupting chemicals. These experiments 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, an *in vitro* human cell culture system will be developed to screen chemicals expected to have estrogenic or antiestrogenic activities.

Concept-Driven Research

In the area of biomaterials, studies will continue to determine if sexual dimorphisms in the immune system could have significant effects on the inflammatory response to implanted biomaterials. The results from this study could be significant for the testing of biomaterials and for elucidating the mechanisms of foreign-body tumorigenesis. As part of the investigations into foreign-body carcinogenesis, experiments will be conducted to elucidate the roles of oxidative DNA damage and cytokine expression that result from the inflammatory response on the initiation and progression of tumorigenesis. Finally, the transgenic tumor promotion study on a variety of biomaterials will continue, with emphasis on tumor type, frequency, and latency. In the area of dietary folate and methyl deficiency, mechanistic studies will focus on methylation dysregulation, abnormal DNA repair, and the incorporation of uracil into DNA during chronic folate/methyl deficiency. In addition, a study will be initiated to ascertain whether methyl-supplemented diets will have multigenerational effects that reduce cancer susceptibility and body weight, while increasing the life span.

New Strategies for the Prediction of Toxicity

During the year, newborn mouse bioassays will continue on specific classes of chemicals including estrogens, antiestrogens, peroxisome proliferators, and lipid peroxidation inducers. Bioassays will be initiated 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. Investigations will also continue to determine the effect of carcinogen structure upon the conformation of DNA and to develop and refine methodologies for the detection, identification, and quantitation of DNA adducts.

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. Thus, while epidemiologic techniques are clearly capable of identifying human carcinogens, these determinations are typically made after the cancer has arisen, which is hardly an ideal situation. And, while animal bioassays are useful for indicating the potential carcinogenicity of chemicals, there are a number of uncertainties concerning the extrapolation of animal data to humans. For example, since only relatively small numbers of animals can be used in bioassays, suspected carcinogens are administered at doses that typically exceed human exposures. In order to assure that the responses detected in the bioassays are germane to humans, a clear understanding of the mechanisms or process of cancer induction in animal models is necessary. This is a major focus of the Division of Biochemical Toxicology.

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. 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 Agency's needs. In addition to providing basic information on toxicological endpoints, such as cancer, these studies serve as the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

A central tenet of cancer research is that tumors arise from cells that have undergone a permanent heritable change in their DNA. Although a number of mechanisms can be envisaged to explain the origin of these heritable changes, clearly a dominant theme in biochemical toxicology research is that, in most instances, they arise from the interaction between chemical carcinogens and DNA to form DNA adducts. As such, the elucidation of the structures of these adducts can provide essential information concerning the metabolic activation pathways of suspected carcinogens. Furthermore, by determining the identity, quantity, and persistence of DNA adducts, insight can be obtained on the effects of the adducts on DNA structure, transcription, synthesis, and repair. Investigations of the specific types of mutations induced by particular DNA adducts can provide a direct test for the role of DNA adducts in carcinogenesis. Finally, DNA adducts can be used as dosimeters to

measure exposure and, thus, provide the data necessary for estimating the relative risk for tumor induction.

While recognizing the importance of exogenous DNA adducts, not all carcinogens induce cancer through their direct interaction with DNA. Additional mechanisms include, for example, perturbations in cell cycle kinetics, the induction or suppression of cell death and proliferation, the initiation of lipid peroxidation with concomitant formation of endogenous DNA damage, and perturbations in DNA methylation which could lead to alterations in gene expression. Clearly, an accurate understanding of carcinogenic risk requires assessing the potential contributions of these factors.

BIOMETRY AND RISK ASSESSMENT

BIOMETRY AND RISK ASSESSMENT

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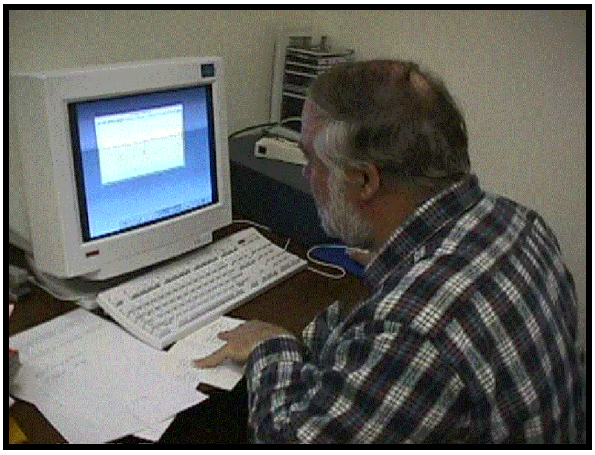

Numerical analysis is essential.

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.

In most cases, unverified 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 Biometry and Risk Assessment program 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. It 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.

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.

FY 98 Goals

1. To develop biometrical methods for estimating risks associated with toxic substances in order to enable setting exposure levels that correctly reflect the underlying uncertainty;

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

3. To conduct research on identifying and quantifying sources of uncertainty in human health risk estimation and to develop methods to reduce the uncertainty;

4. To develop mathematical models for better representation of internal exposure levels and of biological mechanisms in order to improve estimates of the risk of toxic effects;

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

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

7. To participate in interagency risk assessment activities to maintain knowledge of the state of the art, and to improve and unify risk assessment practices across agencies.

FY 97 Accomplishments

Scientists in the Division of Biometry and Risk Assessment had 23 first-authored research papers accepted for publication, and co-authored an additional 17 papers. Major research accomplishments under each of NCTR's strategic research goals were as follows:

Development of Knowledge Bases

Research was conducted 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). An approach was developed for using a logistic regression model to translate short-term results into long-term predictions. Preliminary results indicate 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. For such chemicals it might be possible to eliminate the conduct of a two-year bioassay. The ultimate intent of this research is to provide a means

to accelerate the approval process of human and animal drugs, food additives, and medical devices.

A decision-tree classification method was developed for determining the best sequence for conducting a series of independent diagnostic tests. A likelihood ratio criterion determines the most advantageous testing order, in the sense of identifying which test at each stage will have the best chance of leading to a positive or a negative diagnosis at the next stage or a succeeding stage. A diagnosis is made only after the probability of a positive result attains a predetermined level, high or low, as determined using statistical confidence limits.

New Strategies for the Prediction of Toxicity

A draft manuscript describing a unified approach to safety assessment for both carcinogenic and noncarcinogenic effects was put into a finalized form for publication. 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.

Data collection and evaluation of computerized images of both serial sections of mouse fetuses and laser scanning confocal microscopy optical sections of mouse embryos are underway (E06953.01). Results will be used to reduce the bias in tissue volume measurements for pharmacokinetic modeling, and to produce data to use in growth models for embryonic limb development.

Method-Driven Research

Research was conducted to develop statistical methods for attributing cause of death in animal tumorigenicity studies (E06896.01). It appears to be possible 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. A statistical method for such a partitioning is being developed which could eliminate the need for pathologists to assign a cause of death to each animal in a bioassay. This method can be used to modify the International Agency for Research on Cancer (IARC) cause-of-death test which is widely used to evaluate preclinical data in pharmaceutical development.

A research protocol on statistical tests involving multiple endpoints was written and implemented (E07009.01). A new procedure for adjusting p-values in order to control the family-wise error rate was developed and evaluated in a Monte Carlo simulation study. The procedure was shown to perform well under a variety of conditions.

A computerized optimization procedure was finalized for calculating estimators and tests for the tumor incidence rate in survival/sacrifice experiments (E06870.01). Software has been

developed for implementing statistical tests for multiple-sacrifice experiments and for single-sacrifice experiments and has been made available to the user community.

Concept-Driven Research

A major effort was begun 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).

Investigation of a new biologically based mathematical model for cancer was continued (E06908.01). Because this model depicts the specific mutational events that take place at the nucleotide level, it represents a significant advancement over previous models in the mathematical representation of the cancer process. Several new results were obtained in the context of the new model.

A study was conducted to establish normal blood parameters for the pregnant rat (E06957.11). Data have been collected on a number of clinical chemistry and hematology parameters at multiple time points during pregnancy, and are being evaluated statistically.

FY 97 Interactions with FDA Centers

- Staff members collaborated with scientists at CFSAN on animal growth models and points-to-consider documents for controlling body weight in long-term bioassays.

- Research was conducted on statistical methods for establishing the shelf life of drugs from different batches and packagings (E06909.01). Results were obtained which support FDA's guidelines for estimating the shelf life of pharmaceuticals.

- Staff members collaborated with scientists at CDER on statistical methods for adjusting p-values for multiple testing (E07009.01), and on a points-to-consider document for the analysis of tumorigenicity data in support of pharmaceutical submissions.

- Contact was initiated with scientists at CDER to participate in and to help improve the review process for anti-aging claims for drugs.

- Contact was initiated with statisticians and epidemiologists at CDRH to explore possibilities for future collaborations with those groups.

- A collaborative effort with CFSAN was begun to investigate expanding the Threshold of Regulation for indirect food additives to direct flavor additives.

- Staff members made four presentations at the First Annual Symposium of the FDA Statistical Association.

The division provided statistical consultation on a variety of experiments in support of the divisions of Biochemical Toxicology, Genetic and Reproductive Toxicology, and Molecular Epidemiology. The division also provided oversight to the statistical support group that conducts statistical data analyses for NCTR experiments under the information management contract.

Division scientists, through invited presentations at national meetings, workshops, and universities, have broadened the impact of NCTR's research efforts to improve risk assessment for all types of toxic responses. Staff members have distinguished themselves as conference organizers, committee members, program reviewers, and associate editors of peer-reviewed scientific journals.

FY 98 Plans

All ongoing projects which have not been completed will continue into FY 98. In addition, some current projects will be expanded and several new projects will be initiated, as follows:

Development of Knowledge Bases

Research will continue on the use of a logistic regression model to predict the outcome of the two-year bioassay in rodents, based on the results of short-term tests.

Research will continue on a decision-tree classification model for conducting a sequence of independent diagnostic tests.

New Strategies for the Prediction of Toxicity

A protocol will be developed to study the linkage of physiologically based pharmacokinetic models and biologically based dose-response models in a fully integrated way (X80022). The objective is to improve quantitative risk assessments for chemicals that have sufficient data for fitting such models, by enabling more reliable prediction of risks farther down the dose-response curve.

The Monte Carlo simulation study for the collaborative project with CDER (E06902.01) will be conducted, providing sufficient computing resources are available. Results will be evaluated with respect to the statistical implications of shortened bioassays for assessing the toxicity of drugs.

A joint project with CFSAN to investigate the feasibility of expanding the Threshold of Regulation for indirect food additives to include direct flavor additives will be carried out. This has the potential to greatly reduce the number of flavor additives that would require extensive toxicity testing. In addition, 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 will be pursued.

Research will be conducted on the use of a pharmacokinetic database for extrapolation from animals to humans. The problem of lack of standardization of pharmacokinetic analyses and reporting will be addressed, and a consistent, multi-species pharmacokinetic database for interspecies extrapolation will be compiled. The database will be populated initially with pharmacokinetic data on dexamethasone and methyl mercury.

Method-Driven Research

Work will continue on characterization of the joint action of toxicants, and on risk estimations for mixtures of chemicals (E06984.01). The feasibility of developing a procedure for calculating benchmark doses for chemicals that occur in mixtures will be studied.

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 developed and evaluated. In addition, tests for developmental toxicity studies involving correlated endpoints will be developed.

A protocol will be developed to investigate nonparametric density estimation methods for estimating the distribution of enzyme activity in polymorphic populations (X80021). 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 protocol will be developed to investigate the use of a mixture of normal densities for modeling the effects of enzyme induction on enzyme variant classification.

Research will continue on estimating risks and calculating benchmark doses for nonquantal toxicity data, including developmental neurotoxicity data and data involving mixtures of populations.

An agreement will be pursued with the Radiation Effects Research Foundation in Japan for collaborative research on health risks from *in utero* exposure to radiation. In a related effort, the feasibility of studying the predictivity for humans of radiation effects in rodents will be

investigated, provided it is determined that such a study would be of interest to scientists at CDRH.

Research will be conducted on models for embryonic limb growth and for relating food consumption to body weight in the whole animal. Part of this effort will be done in collaboration with CFSAN.

Concept-Driven Research

Work will continue under E06908.01 on an investigation of molecular dosimetry within the structure of a model that specifically depicts mutational events at the nucleotide level.

Statistical analyses will continue on the series of experiments conducted under the Project on Caloric Restriction (E00501-E00509). Most of the remaining within-study comparisons should be completed in FY 98.

An effort will be made to use the information developed in the Project on Caloric Restriction to assist CDER in developing a method to regulate anti-aging claims for drugs.

Significance to the FDA

The NCTR has been designated by the FDA as the focal point for research in the area of Health Risk Assessment, including investigating the critical assumptions that underlie such 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 key role of identifying uncertainties in the risk assessment process, and developing risk estimation techniques to reduce these uncertainties in order to improve the regulation of natural or synthetic toxic substances occurring in foods, drugs, cosmetics, 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.

GENETIC AND REPRODUCTIVE TOXICOLOGY

GENETIC AND REPRODUCTIVE TOXICOLOGY

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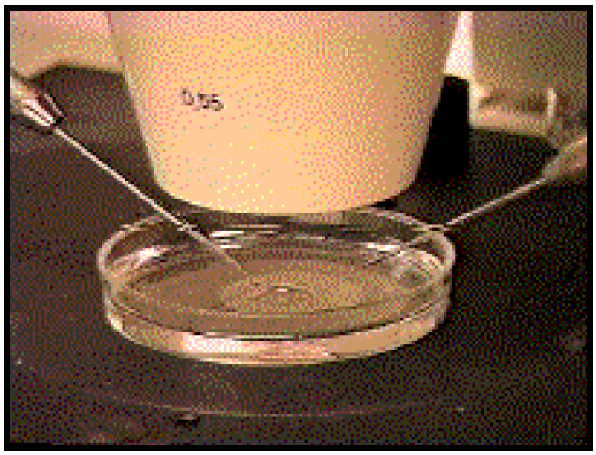
GENETIC TOXICOLOGY LABORATORY

Introduction



Example of micro injection technique to produce transgenic animals.

The FDA requires that petitioners provide data evaluating the potential genotoxic activity of food additives, cosmetics, and human and animal drugs they wish to market. Thus, the 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. Over the years, a number of *in vitro* and *in vivo* test systems have been developed to identify and quantify suspected carcinogens and mutagens. Although much information regarding certain classes of mutagenic carcinogens has accumulated, several recent studies indicate that many of these systems are insensitive to certain rodent carcinogens for which the mode of action is not readily observable. The reasons for the observations are uncertain, but are generally considered to be related to the sensitivity of the specific endpoint evaluated and the relevance of the metabolic activation system used to transform unreactive chemicals to species that react with critical cellular informational molecules leading to a genotoxic response. Alternatively, the chemical of interest may induce the carcinogenic response via mutation by indirectly damaging DNA and causing mutations through a secondary mechanism and not by means of direct covalent adduction to the DNA.

In order to increase our ability to identify potentially hazardous genotoxic and putative nongenotoxic chemicals, to understand their mode of action, and perhaps provide additional test systems when a reduced carcinogenesis protocol is employed, a variety of transgenic *in vitro* and *in vivo* mammalian systems are being developed and validated. Transgenic systems provide *in vitro* and *in vivo* tools which can more closely mimic conditions that exist in the human body, increase the potential for detecting weak carcinogens, and decrease the time required to assess accurately a chemical's capability to induce the carcinogenic process. These systems are being developed with the intent of providing relevant data which petitioners and FDA regulators may review in order to determine the potential carcinogenicity of food additives, cosmetics, and human and animal drugs.

FY 98 Goals

1. Develop and validate sensitive and predictive non-transgenic and transgenic human and rodent *in vitro* somatic systems for the identification and quantification of human toxicants, especially carcinogens and mutagens.

2. Develop and validate sensitive and predictive non-transgenic and transgenic rodent *in vivo* systems for the identification and quantification of human toxicants, especially carcinogens and mutagens.

The approach for the first goal is to utilize relevant *in vitro* systems to evaluate risk to the human genome. In order to accomplish this, the division is using the human lymphoblastoid cell line, AHH-1, 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-chromosomal loci. Also, these systems easily lend themselves to molecular analysis making cross-species comparison possible. An additional *in vitro* transgenic system being validated is a Rat2 embryonic fibroblast cell line that carries stably-integrated copies of a lambda *lacI* shuttle vector. Primary rodent hepatocytes are also used to evaluate toxin- induced alteration in specific and non-specific gene expression.

The approach for the second goal is to use non-transgenic and transgenic mice and rats to evaluate mutagens and carcinogens of interest to the FDA and the NCTR. These *in vivo* systems are expected to complement and perhaps eventually replace the present rodent bioassay because their heightened sensitivity would obviate tests at high doses where cell toxicity and mitogenicity become predominant. Also, molecular alterations can be described in the transgene, an endogenous surrogate gene, and in the cancer gene(s) allowing a more direct comparison to the molecular effects described in humans.

FY 97 Accomplishments and FY 98 Plans

There are a number of protocols ongoing in this research area. Of these, eight were initiated as a result of numerous discussions with fellow scientists and regulators from all of the FDA centers. These protocols directly address the needs stated in FY 98 goals.

During the past year, the division has concentrated its efforts on determining the ability of several chemicals to induce mutations and programmed cell death in the human lymphoblastoid cell lines AHH-1, h2E1v2 (expressing human *CYP*2E1) and MCL5, a cell line transfected with *CYP*1A2, *CYP*3A4, *CYP*2A6, *CYP*2E1 and epoxide hydrolase human cDNAs.

They previously found that chloral hydrate (CH), at cytotoxic concentrations of 50% or less survival, induced mutations both at the *Hprt* and *Tk* loci in the h2E1v2 cell strain but not in the non-transgenic parent CHO cell strain suggesting a requirement for *CYP2E1* expression for genotoxic activity. This drug, which increases endogenous DNA adducts, mainly induced *Tk* mutants indicating that it acts primarily through a clastogenic mechanism. This interpretation was further verified by observing induction of micronuclei, an endpoint that measures cytogenetic damage, in the h2E1v2 cell strain. This division responded to an FDA request to evaluate micronuclei induction by phenolphthalein in MCL5. It found statistically significant induction of micronuclei at the high doses of 5 and 10µg/ml, confirming the *in vivo* induction of micronuclei in rodent erythrocytes, and indicating a potential hazard for the human genome.

Loss-of-function mutations in the *p53* tumor suppressor gene result in an altered response to DNA-damaging agents. Included in the mutant phenotype are the loss of cell cycle checkpoints and delayed apoptotic cell death, characteristics consistently observed in the AHH-1 $Tk^{+/-}$ cell line following exposure to DNA-damaging agents. In order to determine the functional status of the *p53* gene in the AHH-1 $Tk^{+/-}$ cell line, molecular analysis was performed on exons 5-9 of the *p53* gene. Initial single strand conformation polymorphism (SSCP) analysis of AHH-1 $Tk^{+/-}$ revealed an abnormal migration pattern of exon 8 when compared to the control. Subsequent sequence analysis indicated that a base-pair substitution (CGG --> TGG) mutation had occurred at codon 282, a reported "hot spot" for mutations in the human *p53* gene. Neither SSCP nor sequence analysis of MCL5 indicated any difference from wild-type DNA. These results suggest that the lack of a G_1 arrest and the delayed entrance into apoptosis observed in the chemically exposed AHH-1 $Tk^{+/-}$ cells are at least partially accounted for by a loss-of-function mutation in the *p53* gene. Ongoing efforts are attempting to understand the signal transduction pathways responsible for triggering programmed cell death and a protocol has been approved to further evaluate a series of endocrine disruptors, chemicals of interest to the NCTR and FDA, in the AHH-1 $Tk^{+/-}$ cell system. Preliminary evidence indicates that the phytoestrogen genistein induces a significant increase in the number of *Tk* mutant clones with the slow-growth phenotype indicating that it is a chromosomal mutagen. They plan to evaluate genistein in L3, another human lymphoblastoid cell line, which has a wild-type *p53* DNA sequence, to determine whether or not *p53* status affects recovery of these types of mutants.

The division also has initiated approaches to goal #2 by utilizing non-transgenic and transgenic technology to expand the number of endogenous and exogenous reporter genes suitable for detecting *in vivo* mutations. The division is utilizing rodent strains, developed by NCTR, commercially or by colleagues in other institutes, as *in vivo* systems to screen chemicals of interest to the NCTR and the FDA and to understand the processes associated with carcinogenesis. They are determining spontaneous and chemically induced mutation frequency in the Big Blue Transgenic Rat (BlueRat) containing the *lacI* transgene, in an endogenous gene (*Hprt*), and in cancer genes in target organs. Where appropriate, the

mutational spectra of the reporter genes and cancer genes will be evaluated. Initial results comparing mutation frequency and phenotypic expression time of *Hprt* and *lacI* genes in splenic lymphocytes are in progress. The data indicate that there is a dose- and time-dependent response in the *Hprt* and *lacI* gene with *Hprt* fully expressing 10-12 weeks post DMBA exposure followed by a steady decline, while the *lacI* fully expresses 6 weeks post exposure and then plateauing. They also examined the types of mutations induced in these reporter genes and determined that the overall mutation profiles were remarkably similar. The majority of mutations were base pair substitutions, with the most common mutation being A:T-->T:A transversion. Differences were found for the mutational responses in the endogenous gene and transgene with respect to the location of the mutations and the orientation of base pair substitutions in the DNA strands. In most cases, these differences could be explained by the nature of the target genes. The results to date support the use of the *lacI* transgene for detecting *in vivo* mutation.

Two other protocols investigating the utility of the ΦX174 transgenic mouse and the *p53* knockout mouse, using the newborn mouse assay paradigm as tools to evaluate recalcitrant chemicals of interest to the FDA and the scientific community, have been developed. Preliminary data with the ΦX174 transgenic mouse indicate that when this animal is exposed to ENU and mutations in the *Hprt* gene and the ΦX174 *am3* allele in splenic lymphocytes are measured, the *Hprt* forward mutation assay is much more sensitive to this A-T specific mutagen than is the ΦX174 reversion assay. Efforts are underway to increase the sensitivity of the ΦX174 assay and to compare its sensitivity to that of the Big Blue® assay. The *p53* studies are ongoing and results should be forthcoming during FY 98.

To assist the Agency in evaluating transgenic models, the division has generated a mouse embryonic stem cell line in which one copy of the autosomal *Tk* gene was disrupted by homologous recombination. This *in vitro* system has recently been shown to behave similarly to the mouse lymphoma L5178Y and AHH-1 $Tk^{+/-}$ systems for evaluation of ethyl nitrosourea-induced *Tk* mutants. Unlike the *Hprt* locus and the existing *in vivo* transgenic loci, which mainly detect base pair substitutions, frameshifts and intragenic deletions, *in vitro* data suggest that the *Tk* target will also be sensitive to mutations involving recombination and loss of heterozygosity as well as multilocus deletions. These cells have recently been used to produce chimeras, which have been mated to produce offspring carrying the transgene. $Tk^{+/-}$ mice are being backcrossed to C57Bl6 mice to transfer the $Tk^{+/-}$ genotype into the C57Bl6 background. Breeding of $Tk^{+/-}$ resulted in the generation of viable $Tk^{-/-}$ knockout offspring. At the present time, they are expanding the colony of $Tk^{+/-}$ mice for use in *in vivo* mutagenesis experiments that will compare spontaneous and induced mutant frequencies at the autosomal *Tk* and X-linked *Hprt* loci in mouse splenic lymphocytes. Thus, this mouse model may provide an endogenous reporter gene that is sensitive to the major types of mutational events that are significant to human health.

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. These systems are capable of simulating the human condition, increasing the ability to detect weak carcinogens, and decreasing the time required to evaluate a chemical's genotoxic potential. Each FDA center has expressed an interest in and a need to utilize transgenic systems as a toxicity screen and as a model for drug/biological interaction. The development of transgenic systems can also provide a model for identifying biological activity, especially in the assessment of bioengineered products regulated by the FDA. Biotechnology product sales are expected to increase seven-fold during the next five years with over 400 new food products alone entering the consumer market. This will greatly impact FDA's need for validated systems capable of defining exposure and assessing risk.

CALORIE 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 which respond to this paradigm have been developed. It is now important to address two primary issues of interest to the NCTR and FDA: 1) development of 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.

FY 98 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.

FY 97 Accomplishments and FY 98 Plans

Free radical mechanisms are thought to play a role in the aging process. Mechanisms of free radical production during normal metabolism were studied in FY 97. They found that the complexes of the electron transport system (ETS) become dysfunctional, and become more prone to error during aging. The result is an ever-increasing free radical load from mitochondria. This may lead to an accelerated rate of damage to important macromolecules yielding more dysfunctional ETS. They have also shown that detoxification of reactive oxygen species is also compromised during aging, and may contribute to this vicious cycle of events. CR has been shown to significantly reduce the types of damage which accumulate with aging and offsets the time to development of dysfunction of the ETS. Thus, CR may produce its desirable effects by limiting free radical production and maintaining integrity of mitochondria. A study to evaluate the effects of CR on mutagenicity and the role of free radicals in the mechanism of toxicity of bleomycin [a reactive oxygen species (ROS) generator] in rats has been initiated. Preliminary results indicate a significant increase in *Hprt* mutation frequency in splenic lymphocytes of *ad libitum* (AL) fed female rats. There was no increase in mutation frequency at any dose tested in lymphocytes from CR rats. Glutathione peroxidase (Gpx) activity was significantly increased in the liver cytosol fraction of AL bleomycin-treated animals. However, there was no increase at any dose in CR animals. This suggests that bleomycin metabolism produces free radicals leading to the induction of Gpx in the liver. These free radicals may be involved in a mechanism leading to increased mutation frequency in AL rodents. The mechanism through which CR reduces ROS production during bleomycin metabolism is not clear, but will be further investigated in FY 98.

A collaborative study with CFSAN has been initiated to determine the ideal level of caloric intake and to define the impact of caloric intake on animal bioassays. This study is evaluating a number of toxicological endpoints in rodents fed varying levels of calorie restricted diets. The synthetic diets have been identified and purchased and animals placed on test in FY 98.

Human CR studies were initiated during FY 97 using a surgical model. Morbidly obese patients which undergo bariatric bypass surgery experience imposed CR which is nearly identical to our rodent biomarkers program. This model will be used to validate various physiological, biochemical, metabolic and molecular endpoints in humans. Additionally, the work should provide a basis for future human studies which may investigate risk associated with various food additives, health food products (such as antioxidants), anticancer drugs and other similar products. In FY 97, seven patients have undergone bypass surgery. Physiological measures have been made on these patients and preliminary data indicate that variables such as body temperature, oxygen consumption, CO_2 production and respiratory quotient (RQ) in humans respond to CR in a manner similar to that in rodents. Biochemical evaluation of blood constituents and biopsy tissue has begun, but preliminary data are not

yet available. The division plans to enter 15 additional patients to the study in FY 98 to continue adding data from both AL and CR humans which will contribute to the validation of their various endpoints.

Significance to the FDA

Fundamental to the FDA is its legislative responsibility to provide for the product safety of those items for which it has regulatory responsibility. The process of risk assessment has become integral to the establishment of safety guidelines. The assumptions used in the process of risk assessment have given greater uncertainty to such calculations than any other factor. NCTR examination of one such assumption (independency of diet) has led, and is continuing to lead, to major changes in how toxicity is evaluated and assessments conducted.

Less widely recognized and yet of equal importance is the legislative responsibility of the FDA to advise on the composition and amounts of a healthy human diet. This holistic question has been universally approached in the past in a piece-meal fashion due to the complexity and diversity of biological functions impacted by dietary intake. As a result of the program's somewhat unique, interdisciplinary approach, much of its previous work can be used to help establish what is a healthy human diet, especially in regards to caloric intake.

In many respects the efforts of this program not only cut across research areas and disciplines, as well as across agencies (NIH and FDA), but also across the overall goals of NCTR (knowledge bases, new predictive systems, and/or method-, agent-, or concept-driven research). The project was concept based, and in order to test these concepts, new methods were developed and products patented or copywritten. More importantly, the knowledge that was and is continuing to be generated via this approach, while adding to the base of knowledge comprising regulatory toxicology, is now being put to practical use on a daily basis by those interested in toxicology testing for regulatory purposes.

Finally, in experimental biological sciences, the ultimate test is the relevance of findings in lower animals to human health. The study initiated in FY 97 in collaboration with the University of Tennessee, Memphis, will determine whether or not the findings made previously at NCTR are or are not relevant to similar events in dietary restricted humans.

REPRODUCTIVE TOXICOLOGY LABORATORY

Introduction

Dr. Randy Streck is loading retinoic acid probes for receptor genes on tissue slices.

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.

FY 98 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 new concepts on how xenobiotics produce developmental toxicant effects.

3. 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), their binding proteins, and receptors. Since diabetes increases the risk of birth defects even in women on insulin therapy, these molecular probes may be important in the etiology of such birth defects.

FY 97 Accomplishments and FY 98 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 Issues 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. Two papers on Quantitative Structure Activity Relationships

(QSAR) models for estrogen 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). Additionally, scientists within the laboratory are collaborators with Dr. Fred vom Saal (University of Missouri - Columbia) in a research project on endocrine disruptors.

Center studies have characterized the effects of two newer anti-estrogens, droloxifene and toremifene, on developmental endpoints in the rat uterus. The results for toremifene are compared to those previously described for the related drug tamoxifen in a submitted manuscript.

Three published papers describe continuing work on the developmental effects of phytoestrogens. Papers from the Third International Phytoestrogen Conference will be published in the March, 1998 Proceedings of the Society for Experimental Biology and Medicine.

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.

A classical Segment II teratology study of fumonisin B_1 (FB_1) in rabbits was completed. Decreased fetal body and organ weights were observed but only at the higher FB_1 doses. These doses also produced a significant amount of maternal toxicity, so it is unclear if the effects on fetal weight were secondary responses to maternal toxicity. The ratio of sphingonine to sphingosine, which was used as a biochemical marker of FB_1 exposure, was increased in a variety of 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. A final report has been submitted and accepted, and a manuscript describing this work is in press.

The anticonvulsant drug, carbamazepine, is believed to produce neural tube defects in approximately 1% of exposed offspring. The drug does produce developmental toxicity in animal models, and it is also capable of producing neural tube defects in a rodent whole embryo culture system in which rodent embryos are cultured directly in serum containing carbamazepine. The laboratory scientists have hypothesized that the putative teratogen is not the parent compound but instead is a reactive metabolite that is formed during metabolism of the drug. They further hypothesized that this reactive intermediate could be detoxified by the tripeptide glutathione and was possibly formed by co-oxidation of carbamazepine by the prostaglandin H synthase enzyme. They have manipulated embryonic glutathione levels by use of the inhibitor buthionine sulfoximine and the precursor N-acetyl

cysteine. These compounds had the desired effects on embryonic glutathione levels but were unable to alter the incidence of carbamazepine-induced embryotoxicity. Further work with acetyl salicylic and indomethacin, inhibitors of prostaglandin H synthase, have also failed to alter the incidence of carbamazepine-induced embryotoxicity. These results suggest that carbamazepine is not co-oxidized by prostaglandin synthase, and if a reactive intermediate is the actual developmental toxicant, it is not detoxified by glutathione. These results are being collated for submission for publication during FY 98.

Another 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 the vitamin, folic acid may be involved. In humans, supplementation with folic acid has been demonstrated to decrease the recurrence of neural tube defects. One of the biological roles of folic acid is in the synthesis of methionine which is the precursor in the synthesis of S-adenosylmethionine. The latter compound is the methyl donor for a number of methylation reactions. The laboratory postulated that VPA might decrease embryonic S-adenosylmethionine levels leading to decreased methylation reactions (especially decreased methylation of DNA); this, in turn, can result in alteration of gene expression. They have preliminary evidence that VPA decreases both embryonic S-adenosylmethionine levels and genomic DNA methylation. During FY 98, they plan to confirm their earlier observations and to examine DNA methylation following treatment of embryos *in vitro* with other compounds that produce neural tube defects. They also 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). Recently, immunochemistry 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 laboratory has investigated the role of stress protein synthesis in the embryotoxicity produced by retinoid acid. This drug produces cleft palate and limb defects when administered to pregnant mice during organogenesis, and it is a derivative of 13-cis-retinoic acid which is known to produce birth defects in humans. This compound leads to the synthesis of a set of proteins known as heat shock or stress proteins. The role that these proteins play in developmental toxicity is unknown, but they have previously shown that they are preferentially synthesized in tissues which are later shown to be malformed by the drug. They are not synthesized in tissues that are insensitive to the developmental toxicant properties of the compound. The purpose of the present experiments is to determine if there is a dose-response relationship between stress protein synthesis and malformations. Preliminary results suggest that there are dose-response relationships for both stress protein synthesis and malformations. During FY 98, the laboratory plans to complete these experiments and to continue developing a method to quantitate gel autoradiographs.

Folic acid has been demonstrated to decrease the recurrence 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. During FY 98, they plan to investigate the role of some enzymes involved in folate metabolism in producing neural tube defects using antisense oligonucleotide knockout of these genes using an *in vitro* embryo culture method; further, they will evaluate the role of the environment in overcoming these knockouts by adding folic acid to the embryos in culture.

A molecular biology capability has now been brought to this research area which enables developmental toxicity to be measured in terms of effects on fetal gene expression. This approach has been applied to determine the effects of maternal insulin-dependent diabetes on fetal expression of eight insulin-like growth factors (IGFs) and binding protein mRNAs. The laboratory has been able to identify a single binding protein, previously demonstrated to function as a growth inhibitor in other systems, that is up-regulated in the growth retarded fetuses of insulin-dependent diabetic dams and down-regulated by insulin treatment. The extent of regulation of this binding protein gene has now been quantitated by extremely precise molecular techniques. This molecular approach will now be extended to identify biomarkers of developmental toxicity associated with maternal non-insulin-dependent diabetes and with treatment with the antidiabetic drug thiazolidinedione.

Estrogens are developmental toxicants, and some effects of estrogens are thought to be mediated by members of the IGF system. Therefore, from the early fetal stage through mature adults, they have characterized expression in the developing uterus of IGF, IGF binding protein and IGF receptor mRNAs. They have also begun to analyze the extent to which estrogens and anti-estrogens alter uterine growth and development through direct effects on the expression of molecules in the IGF system.

Based on gene knockout experiments, teratogenic effects of retinoic acid on limb skeletal growth and morphogenesis were known to be mediated through a specific retinoid receptor species, Retinoid X Receptor-α (RXRα). Yet the expression patterns of the RXRs (α, β and γ) had not been characterized for the stage at which limb skeletal patterning is established, and RXRα was reported to not be expressed in the skeleton at any stage. Therefore, they characterized expression of all 3 RXRs throughout limb development, and discovered that RXRα is strongly expressed in the fetal limb skeletal precursors, consistent with the knockout results.

Based on previous work in this laboratory, fetal growth of specific brain regions (and development of the behaviors they control) are known to be exquisitely sensitive to maternal retinoic acid exposure. Therefore, they tested for altered expression of retinoic acid-regulated growth factors within those regions of the fetal brain. No obvious qualitative

changes in growth factor expression have been detected, but more sensitive techniques are currently being used to detect more subtle quantitative changes.

Investigations utilizing a laser scanning confocal microscope coupled with a Silicone Graphics Workstation have electronically imaged mouse embryos. The 3-D reconstruction of gestational day 9 to 11 embryos has provided insight into logistical problems of gestational age identification markers, processing shrinkage of tissues and organs, and storage, identification, and retrieval of the vast amounts of electronic data being generated. This next year will continue this effort to collect developmental data on normal embryonic and fetal development in the mouse and rat.

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 AND CHEMISTRY

MICROBIOLOGY AND CHEMISTRY

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Animal health surveillance programs are crucially important in rodent disease prevention and include microbiological monitoring of animal feed, bedding and water.

MICROBIOLOGY LABORATORY

Introduction

Microbiology is an exceptionally broad discipline encompassing research areas as diverse as taxonomy, physiology, biochemistry, 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.

FY 98 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 in normal populations and in patients with intestinal disorders.

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 and food additives; 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 coating 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 which 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.

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 which 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) developing bacteriological assays for determining chemicals, such as folate in culture fluid, for research projects within NCTR; 3) culturing and identifying microbial contaminants for other projects and programs within the NCTR and other FDA centers; and 4) developing and testing new methods in diagnostic microbiology for other FDA centers.

FY 97 Accomplishments and FY 98 Plans

In FY 97, 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 and exchange of scientists between laboratories. 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 Division of Microbiology and Chemistry at the NCTR 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 a result. Scientists in the laboratory are attempting to implement molecular methods to both improve the sensitivity and accuracy of the test, and shorten the time required for a definitive answer. One such approach is the use of a mycobacterial strain carrying the firefly luciferase gene to determine the number of bacteria that survive exposure to the disinfectants (E-06965.01). Surviving bacteria produce light upon the addition of luciferin, allowing quantitation of the disinfectant activity. By developing and validating such an assay, they hope to be able to rapidly assess the effectiveness of both currently available disinfectants and future products.

Three FDA analysts from Denver, Minneapolis, and Winchester Engineering and Analytical Center (WEAC) were trained in the AOAC tuberculocidal assay by scientists at the NCTR. The topics covered were biosafety and facility requirements, contamination control, growth and standardization of the test organism, media preparation, carrier preparation, disinfectant preparation and neutralization, phenol standardization, test performance, and interpretation of results. Discussions also included how their current facilities could be modified to provide appropriate conditions for the test.

2. Develop molecular and mass spectrometry 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 and Chemistry in FY 97 had a project (E-6988) to develop molecular methods to detect and identify foodborne bacterial pathogens. In addition, scientists in the Microbiology Laboratory collaborated with scientists in the Chemistry Laboratory to use mass spectrometry methods for the rapid identification of bacteria (E06785.00 and E06931.00).

A protocol (E-6988.01) for the detection of 13 species of foodborne pathogens in foods using the polymerase chain reaction (PCR) technique was developed in FY 97. 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.

The survival of *Shigella* species on prepared foods which 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 which can be severe and even life-threatening. Because *Aeromonas* can resemble *E. coli* on several media, the incidence and the importance of this microorganism has 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 developed in FY 97 for the detection of *A. caviae* and *A. trota* from seafood and water samples in less than eight hours.

Little is known about the aerolysin toxins and their regulation. The molecular cloning of the aerolysin (toxin genes) and nucleotide sequences of these genes will allow the development of a specific probe for toxin-producing strains and the study of the structural and regulation of these genes in pathogenic organisms. In order to determine the mode of action of bacterial toxins on cell lines, antigenic properties, and for the development of immunodiagnostic kits against these toxins it is important to have highly purified toxins. They will develop in FY 98 a rapid method for producing pure toxins by cloning various toxin genes onto 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 (E-7001.01) has been initiated with Dr. Angelo DePaola, FDA, Gulf Coast Seafood laboratory (GCSL) staff, Dauphin Island, AL. Dr. DePaola of 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 Center for Disease Control (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 this/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.

A rapid method to detect low levels of *V. vulnificus* from seafood samples will be developed in FY 98 by the construction of luciferase reporter bacteriophage. A broad host lytic phage will be isolated and will be genetically characterized. A green fluorescent protein or Lux gene reporting system will be developed to screen pathogenic *V. vulnificus* species in seafoods. The results obtained from these projects will be used by GCSL or NCTR for the development of detection of *V. vulnificus* from seafood and validate effectiveness by comparison with accepted methods.

A new method, using matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF) mass spectrometry, to identify intact whole bacteria that are found as contaminants in foods is being developed. Some of the results, for selected potentially pathogenic Gram-negative aerobes and facultative anaerobes, have recently been published in *Rapid Communications in Mass Spectrometry.* Since the mass spectrometric method has been found to be less reliable for seven-day-old bacterial cultures than for fresh cultures, improvements in the method to allow the analysis of older bacterial cultures are now being investigated.

3. Assessing the effects of food additives and drugs in food on the human intestinal microflora. Determining 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.

The research program in the Microbiology Laboratory at the NCTR in FY 97 has developed molecular methods for the detection of predominant anaerobic bacteria in human and animal fecal samples. PCR procedures based on 16S rRNA gene sequences were developed and used for quantitative detection of intestinal microflora in human (adult and baby) feces and animal (rat, mouse, cat, dog, monkey and rabbit) feces. This method, including the fecal sample preparation method, is rapid and eliminates the DNA isolation steps. The method is being used in research at the NCTR for assessing the effects of food additives, antimicrobial residues and caloric restriction on the human intestinal microflora. In addition, Microbiology staff have been contacted by scientists from universities, pharmaceutical industry, and regulatory agencies for advice and training concerning the method.

Studies have continued in the laboratory on the determination of the role of intestinal microflora in xenobiotic metabolism. 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 nitroreductase 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 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.

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 microbiologists 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 (E-6901.01) 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 (vibrio) in aquaculture. Currently, antibiotic erythromycin is under FDA's review for approval for its use in salmon and trout culture, specifically for control of bacterial kidney disease. Since aquaculture waste water 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 very limited studies have reported on the environmental fate of erythromycin used in aquaculture. The extensive review of antibiotics used in fish farming systems also led them to write a chapter on the environmental fate of antibiotics for an upcoming book on Bioremediation: Principles and Practice (in press). 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 *Xanthomonas* species has shown that the isolated species is a new strain and, therefore, is named as *Xanthomonas* sp. NCTR.

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 a host of metabolites produced lack antimicrobial activity.

Erythromycin is a drug of choice for the treatment of many diseases affecting animals and poultry. As such, animals and poultry production units have been suspected as a potential reservoir of antibiotic determinants. Although numerous investigations have been undertaken to isolate and characterize the bacterial flora resistant to these drugs, very little information is available on the genetics of these determinants. The scientists in the laboratory targeted the poultry industry where erythromycin has been prescribed extensively (400 G/ton of feed) for the control of Staphylococcosis caused by *Staphylococcus* sp. Forty-six (46) *Staphylococcus* sp. resistant to very high concentrations of erythromycin (256 µg/mL) were isolated from diseased chicken. Thirty-

four (34) of these isolates were identified as coagulase positive *S. aureus* and twelve (12) were identified as coagulase negative *Staphylococcus* sp. Twenty-six (26) of the thirty-four (34) *Staphylococcus aureus* amplified with a pair of oligonucleotide primers specific for the detection of the erythromycin resistant methylase (*ermC*) gene, to produce a 520-bp gene product. Twelve (12) coagulase negative *Staphylococcus* sp. were also *ermC* positive. The presence of the gene was further confirmed by Southern hybridization. A 2.5 Kb plasmid was found to encode *ermC* gene in all *S. aureus* and this plasmid hybridized with the 3.7Kb pE194 that encodes *ermC* gene in clinical strains of *S. aureus*. Their study indicates extensive homologies between these two plasmids. However, none of the plasmids (16.0-2 Kb) of the coagulase negative *Staphylococcus* sp. hybridized with pE194. This suggests that the *ermC* in coagulase negative *Staphylococcus* sp. may be encoded by a megaplasmid (>16.0 Kb) or the gene may be coded by the chromosome. Their current investigations unambiguously indicate the avian *Staphylococcus* may serve as a potential reservoir of *ermC* gene. Characterization of the 2.5 Kb plasmid from avian *S. aureus* and localization of the gene encoding *ermA* and *ermC* will be determined in FY 98.

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 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 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 yanoikuaye* B1 and *Mycobacterium* sp. PYR-1 (protocol E-6999) *S. yanoikuaye* 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.

N,N-dimethylformamide (DMF) is a widely used industrial solvent that is hepatotoxic. Because it is a strong and toxic solvent, it is important to remove all DMF from contaminated waste waters. Although DMF is a simple 3-carbon compound, it is not readily degraded by microorganisms. Several strains of DMF-degrading bacteria were successfully isolated from contaminated soils and the strains were characterized. The bacteria were members of the genus *Hyphomicrobium* and had a high affinity for DMF. The bacteria were thus capable of removing DMF to undetectable levels in aqueous systems containing DMF concentrations as high as 140 mM.

5. Develop 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 FY 97 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.

Several drugs have been investigated recently to determine the metabolic pathways used for their biotransformation by fungi used as microbial models. Cyclobenzaprine, an antidepressant drug, was metabolized to 2-hydroxycyclobenzaprine, *N*-desmethyl-cyclobenzaprine, cyclobenzaprine trans-10,11-dihydrodiol, *N*-desmethyl-2-hydroxy-cyclobenzaprine, 3-hydroxycyclobenzaprine, and cyclobenzaprine *N*-oxide. Cyproheptadine, an antihistamine drug, was metabolized to 2-hydroxycyproheptadine, 1- and 3-hydroxycyproheptadine, cyproheptadine 10,11-epoxide, *N*-desmethylcyproheptadine, *N*-desmethyl-2-hydroxycyproheptadine, cyproheptadine *N*-oxide, and 2-hydroxycyproheptadine *N*-oxide. Cytochrome P450 monooxygenases have been shown to be involved in the metabolism of cyclobenzaprine and cyproheptadine by both fungal and mammalian systems. Phenothiazine, a veterinary anthelmintic drug, was metabolized to phenothiazine sulfoxide and phenothiazin-3-one. The fungal metabolites are generally identical in structure, if

not necessarily in relative amounts, to mammalian metabolites. Current studies are in progress to investigate the metabolism of two additional antidepressant drugs, amoxapine and doxepin, and the heterocyclic contaminants cinnoline, isoquinoline, phenanthridine, phthalazine, quinazoline, and quinoxaline. Large amounts of metabolites identical to those produced in mammalian systems can be produced by the fungal biotransformation system.

Significance to the FDA

The Microbiology Laboratory of the Division of Microbiology and Chemistry 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 interests; 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; 7) sensitive methods for the detection of genetically modified microorganisms; and 8) 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 microbiology.

CHEMISTRY LABORATORY

Introduction

A Chemistry Investigator injects a rat plasma sample through the liquid chromatograph for analysis of estrogenic phytochemicals and metabolites using electrospray mass spectrometry.

Enforcement of regulations requires sound, reliable and validated analytical procedures as the basis of those 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 to determine antibiotic residues in poultry, fish, beef, and milk; antimicrobial/antifungal residues in fish; and, identification of bacteria using mass spectrometry techniques. 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 the dosage form and dosage certification for chemicals scheduled for toxicological evaluation under the National Toxicology Program. These include fumonisin B_1, chloral hydrate, urethane, leuco-malachite green, malachite green, genistein and methoxychlor.

The development of analytical methods to support FDA's regulatory and enforcement actions extend beyond the scope of traditional analytical chemistry. Bioanalytical chemistry provides evidence regarding the bioactive/bioavailable form of regulated compounds, as well as evidence regarding mechanisms of action, individual susceptibility, and potential avenues to minimize the risks associated with toxicants. Research in hardware engineering 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 wide 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.

FY 98 Goals

Bioanalytical Chemistry

Several experiments are planned related to the endocrine disruptors program at NCTR. The laboratory hopes to develop an LC/MS method for genistein-protein adducts, isoflavones and conjugates in rodent feed, and rodent and human blood. The possible formation of autoantibodies to thyroid proteins as a result of genistein feeding will be determined.

Their preliminary use of immuno affinity chromatography will be expanded as an on-line IAC-LC/MS method is used for the quantification of etheno DNA adducts.

Multi-Residue Methods Development

They will obtain incurred tissues from 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 SFE will be investigated as a potential extraction method. The NCTR-developed methodology for the determination/confirmation of malachite green and leuco-malachite green will be modified for use on various fish tissues. Methods for analysis of erythromycin A residues in catfish and salmon tissue samples will also be validated.

New methods work will begin in the area of the active ingredients in dietary supplements. This includes the determination of salicin and related compounds in botanicals (e.g., willow bark) as well as methods for speciation of metals, including Cr, Mn, and As in nutritional supplements.

New SFE and solid phase extraction (SPE) cleanup methods for analysis of nitrosamines in corned beef and dried beef will be developed.

After receipt of incurred hair samples from CVM, the method for detection of beta-agonists in hair will be validated. Unknown beta-agonists in eye samples from the USDA/FDA regulatory screening program will be identified and quantified.

Hardware Engineering

Performance tests necessary to evaluate the realistic potential of the universal interface for use in FDA regulatory and commercial applications involving MS, CRIMS, and perhaps TEA will be conducted under the recently approved CRADA.

A system for encoding bacteria spectra into a format suitable for library searching or pattern recognition will be developed. A specific method for the library search or pattern recognition applications will be selected.

A private-sector partner will be sought to facilitate the commercialization of the fish-freshness indicator test-strip. Development and validation for regulatory and consumer applications will continue.

NTP Support

Ongoing or recently completed compounds will then include chloral hydrate, malachite green, genistein, methoxychlor, fumonisin and fumonisin derivatives. New compounds for study in FY 98 will include nonylphenol, ethinyl estradiol and vinclozolin. For each compound investigated, methods for the analysis of the NTP analyte will be developed in the appropriate matrix, along with dose certification and homogeneity studies for each compound under study.

A method for quantification of urethane in rodent blood will be validated and used to quantify blood levels from a range-finding study.

Surveillance Activities

The Analytical Laboratory Information System (ANALIS) system for data entry will be replaced by a new Laboratory Information Management System (LIMS). SPE methodology will be incorporated into the surveillance laboratory operation.

FY 97 Accomplishments

Bioanalytical Chemistry

During FY 97, the Chemistry Laboratory elucidated the mechanism responsible for the anti-thyroid action of malachite green and related triphenylmethane dyes. This work had increased their understanding of the relevance to human health of rodent bioassay experiments. The mechanisms for inhibition of the coupling reactions by anti-thyroid chemicals and the anti-thyroid action of genistein were also elucidated. A liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method for monitoring exposure to anti-thyroid components in the diet of rodents was investigated. Such a method could also be used to examine the diet of humans for such compounds.

They investigated and prepared immunoaffinity cartridges for use in on-line sample preparation of etheno DNA adducts. This new approach to the enrichment of samples with the rare modified

DNA (adducts) from samples containing very low levels of DNA damage provides an important adjunct or alternative to existing techniques.

Multi-Residue Methods Development

They have worked to develop a number of multi-residue methods, primarily involving antibiotics. In the development of analytical methods for amoxicilin and lincomycin, a bridging study with FDA Denver District was completed and data was submitted for a Center for Veterinary Medicine (CVM) methods trial. 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. An accompanying atmospheric pressure ionization/mass spectrometry (APCI/MS) confirmatory procedure was also developed for 13 analytes.

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 validated and LC/MS methods for confirmation of this antibiotic in chicken liver are being developed.

Non-invasive methods to quantify and confirm beta-agonists in hair were developed using LC-APCI/MS. These methods may provide U.S. Department of Agriculture or FDA an alternative approach that can be applied without harming the animal under study. In a similar manner, such techniques could be applied easily to human subjects for these or related compounds.

Methods for the analysis of volatile and non-volatile N-nitrosamines in cosmetics, nitrite-cured meats, and tobacco products were developed. Claims made to FDA regarding the existence of a low-nitrosamine tobacco resulting from a 'low-nitrosamine' processing method confirmed that the appropriate blind coded tobacco samples did indeed have substantially lower levels of two nitrosamines implicated as human carcinogens. In a related experiment, a liquid chromatography-particle beam-thermo energy analyzer (LC-PB-TEA) for the analysis of non-volatile nitrosamines and tobacco nitrosamines was modified and updated.

Hardware Engineering

A Cooperative Research and Development Agreement (CRADA) was approved to investigate the commercialization of a 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.

A patent application involving rapid identification of whole bacteria based on spectral patterns using matrix assisted lazar desorption ionization/time of flight (MALDI/TOF) MS was submitted

through FDA based on the discovery and first publication of this new method for bacterial identification. The method involves the detection of proteins sampled from intact bacteria without isolation/cleanup.

A patent application was sent to FDA for a color-based fish freshness indicator test-strip 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. Important preliminary findings regarding the measurement of drug purity and enantiomeric composition of drugs by NMR were made.

NTP Support

The compounds investigated this year included urethane, chloral hydrate, malachite green, genistein, methoxychlor, fumonisin and fumonisin-related derivatives. Typically they investigated or developed methods for the analysis of the NTP analytes in the required matrix (i.e., feed). Where possible they adapted an existing method to meet the sensitivity needed for the study. They initiated dose certification and homogeneity analyses for each compound under study.

Surveillance Activities

Automation of screening assays has been increased by the addition of an automatic distiller for protein analyses. Investigation of supercritical fluid extraction (SFE) for fat, vitamin, and pesticide extraction methods has continued but is not yet complete. An LC/MS method for determination of fumonisins in feed was validated and implemented. Similarly, a method for urethane in feed was also validated and showed minimal concentrations of background urethane in the NCTR's animal diet.

Significance to the FDA

The development and validation of relevant analytical methods will enable Agency field chemists 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 bioanalytical 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, bioanalytical chemistry will provide evidence

regarding the bioactive, 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 significantly.

MOLECULAR EPIDEMIOLOGY

MOLECULAR EPIDEMIOLOGY

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INTOX graduate student, Rick Wiese, is shown working on the role of cyclooxygenases in drug and carcinogen metabolism.

Introduction

The strategic goals of the division are: 1) the development, validation, and clinical application of molecular biomarkers of individual susceptibility and of carcinogen exposure; 2) the extrapolation of results from animal mechanistic studies and animal bioassays to humans; 3) the development and validation of "alternatives" to the standard rodent bioassay for assessment of human carcinogenicity; and 4) the international harmonization of these efforts to provide an estimate of individual risk assessment. 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, this research is expected to provide 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. The division's research focus is on the food-borne heterocyclic amines, aromatic amines, and polycyclic aromatic hydrocarbons; and 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 either ongoing or planned for 1998.

The division's experimental approach and project areas are:

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 food-borne carcinogens.

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) Effect of antioxidant supplementation on chloral hydrate-induced oxidative stress in children.
   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 colo-rectal cancer: role of dietary heterocyclic amines.

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

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

Human biomonitoring and DNA adduct detection:

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

Extrapolation between animal studies and human populations:

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.

International efforts in molecular epidemiology, DNA adduct biomonitoring, and biotechnology:

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

9. International harmonization efforts on DNA adduct biomonitoring and its application to risk assessment.

10. Development and validation of DNA chip technology for human diagnostics.

FY 98 Plans and Goals

During 1998, the division will continue with the projects described under the section, "1997 Accomplishments" but with additional 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.

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 colo-rectal cancer susceptibility. The mechanisms that control gene expression will be examined, including analysis of genetic variants as well as changes in gene methylation, an epigenetic mechanism.

Polymorphisms of cytochrome P450 1B1 and tissue-dependent expression. Studies on cytochrome P450 1B1 (*CYP1B1*), which 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 we have developed and are using for immunohistochemical studies on tissue localization in humans. The recent isolation of microsomes from a human lymphoma that selectively expressed *CYP1B1* will provide a abundant source of enzyme to conduct functional assays. In addition, their 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, 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, with special emphasis

on the role of cytochrome P450 2A6 (*CYP2A6*), which like cytochrome P450 2E1, is involved in the metabolism of carcinogenic nitrosamines that are often contaminants of the food supply.

Polymorphism of estrogen sulfotransferase. Estrogen sulfotransferase (*SULT1E1*), which is present in human endometrium and appears to be a critical pathway leading to the formation of tamoxifen-DNA adducts, exhibits polymorphic expression (see 1997 Accomplishments). Using a specific anti-peptide antibody and measurements of *SULT1E1* mRNA, they will examine this polymorphism and begin sequencing and/or coding 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 women receiving this anti-estrogen as a treatment for breast cancer.

Polymorphisms of glutathione *S*-transferases A1, A2, and P1. 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 food-borne heterocyclic amines and mycotoxins. Since they have already measured the levels of these proteins in 30 human liver cytosols, they will soon begin sequencing the 5'-regulatory region of the genes, which are purported to be only 150-200 bases upstream of the coding region. Again, if this approach is successful, then 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. Likewise, GST P1, which is the major detoxifying enzyme in extrahepatic tissues, has been shown by the division and other laboratories to exhibit three variant alleles, GST P1*A, P1*B, and P1*C, representing frequencies of about 60%, 35%, 15% in the general population. In 1998, they will purify these enzymes from genotyped tissues to examine their activity toward carcinogenic substrates, as well as chemotherapeutic agents. Moreover, their development of genotyping assays for these alleles will be applied to a variety of molecular epidemiological studies to determine whether or not this polymorphism affects cancer susceptibility or response to chemotherapy.

GST P1 is the major GST in human bone marrow and can serve both to detoxify carcinogens and to conjugate chemotherapeutic agents. Thus, they are planning a collaborative case-control study with investigators at the Mayo Clinic 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), the division is evaluating the role of genetic polymorphisms in GST M1, GST P1, GST A1 and GST A2 in response to therapy, as well as to levels of glutathione 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 1998, they will also extend their examination of human polymorphisms to DNA repair enzymes by using the host reactivation base excision assay with cryo-preserved 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 colo-rectal cancer and they are in the process of establishing this assay in our 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.

Substrate specificity and activity of COX-1 and COX-2 toward metabolic activation of food-borne carcinogens. A new project area will examine the human cyclooxygenases, COX-1 and COX-2, and their role in carcinogen bioactivation, especially in relation to colo-rectal 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 food-borne carcinogens and determine their levels 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. These data are expected to aid FDA in the drug approval process and should provide additional insights into the etiology of metastatic colo-rectal cancer.

2. Chemoprevention.

Coffee and tea effects on *CYP1A2*, GSTs, and *N*-acetyltransferases. 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, our data showing that the coffee lipids, kahweol and cafestol, not only induce GSTs but also appear to down-regulate *N*-acetyltransferases (NAT1 and NAT2), effectively changing the phenotype of rats from rapid to slow acetylators, will need further validation. In collaboration with the Division of Genetic

and Reproductive Toxicology, this division will use rat and human hepatocytes to explore the mechanism of NAT gene regulation. Also planned are 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 and on the impact of tea and coffee consumption on drug bioavailability and on colon and bladder cancer risk by food-borne carcinogens.

Effect of antioxidant supplementation on chloral hydrate-induced oxidative stress in children. The division is beginning a pilot study in collaboration with Arkansas Children's Hospital to determine whether or not this anesthetic, which is commonly used in pediatric medicine and reported to be carcinogenic in animal bioassays through induction of lipid peroxidation, also induces oxidative stress in children. The division plans to characterize and quantify lipid peroxidation products in the urine of pediatric patients who will be receiving a single dose of chloral hydrate during minor surgical procedures (phase I). Physicians at the hospital are presently recruiting pediatric patients for the first phase of the study, which will be used to design the second phase of the study involving measurement of these urinary biomarkers before and after treatment with single or multiple doses of chloral hydrate. In phase II of the study, subjects will be divided into groups receiving a placebo for one month prior to treatment and those receiving antioxidant supplements including vitamins A, C, and E, which are known to effectively inhibit lipid peroxidation and oxidative stress in animal models. These efforts may lead to public health recommendations on the use of antioxidant supplements in preventing chloral hydrate-induced toxicity in children, thus supporting FDA's post-market surveillance of this drug in sensitive subpopulations.

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. Recent improvements in S adenosylmethionine (SAM) and homocysteine analyses brought about by division personnel will permit the use of non-invasive techniques to examine possible interactive effects between diet, genetic polymorphisms, and compound exposures on the methylation status of individuals. Preliminary results within the division have been encouraging in this regard (*cf.* 1997 Accomplishments), and it is expected that an interagency agreement (IAG) with the National Cancer Institute (NCI) will be established to examine this question. Two genetic polymorphisms centered on abnormal methyl metabolism, catechol *O*-methyltransferase (COMT) and methylenetetrahydrofolate reductase (MTHFR), have been recently found to be associated with altered cancer risk for breast cancer and colo-rectal cancer, respectively. Their impact on methylation status will thus be examined. Other laboratories are planning collaborative studies with the division to compare methylation status in humans and cancer susceptibility. One such collaboration will examine possible defects in the regulation of the enzyme, DNA methyltransferase, and the development of human colon cancer. Preliminary studies indicate that a number of toxic or carcinogenic agents appear to alter the activity mediated by this enzyme, *e.g.*, cadmium, arsenic, and fumonisin B_1. 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 colo-rectal 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 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.

The Division of Molecular Epidemiology is also planning to conduct a straightforward bioassay of homocyste(i)ne toxicity. Oddly, despite homocysteine's increasingly recognized role in atherosclerosis and its known role in producing a hypomethylating environment, the compound itself has never been adequately investigated as an etiologic agent either in heart disease or in cancer. This study would consist of a 120-day feeding study with DL-homocystine and would examine pathological effects on five different tissues in rats. If results warrant, a chronic animal bioassay or appropriate human pilot study will be proposed.

3. Etiology of human colo-rectal cancer: role of dietary heterocyclic amines.

In its ongoing study of dietary and genetic factors related to risk of colo-rectal cancer, the division will continue to genotype or phenotype cases and controls for *CYP1A2, NAT2,* and *SULT1A1* activity. It will also apply the DNA repair assay using cryo-preserved lymphocytes from the study participants, and plans 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 in polyp recurrence, and the possible modulating effects of NAT1 and NAT2 polymorphisms on risk associated with dietary consumption of heterocyclic amines.

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

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 pre-menopausal breast cancer, the Public Health Service and the 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 will be collected.

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 is conducting 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 commonly eaten in this region, but are not included on standard food-frequency questionnaires in the U.S. Over 150 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. These data are likely to be extremely important for future studies on the role of diet in disease etiology in African-Americans.

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 analysis of molecular epidemiological data from its case-control study on pancreatic cancer which, like colo-rectal cancer, shares common risk factors that suggest the role of food-borne heterocyclic amines, including high meat and energy consumption, and low intake of cruciferous vegetables and fruits. Together with the data on pancreatic DNA adducts as described in "1997 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 1998 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.

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

Another major emphasis in 1998 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 1997 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 1998, the division will use ^{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. A study of chemical carcinogenesis in human breast epithelial cells, and of the effects of environmental and drug exposures and genetic susceptibility on these processes, is 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 their 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 relationship to environmental exposures, as well as to polymorphisms in genes involved in metabolism of dietary and environmental carcinogens and endogenous steroid hormones.

The division is also beginning to focus on the role of metabolism of steroid hormones in carcinogenesis in other hormonally responsive tissues. This includes a study of estrogen metabolism in human ovarian tissues and of the effects of hormonal regulation in combination with genetic variability on these processes. Identification and characterization of estrogen-specific biotransformation pathways in normal tissues will enable the dissection of events that participate in the generation of, and/or the protection from, the production of highly estrogenic or DNA-damaging metabolites. Specifically, the study will: 1) identify the major metabolic enzymes acting on estradiol in the human ovary as a function of hormonal exposure associated with ovulation; 2) characterize metabolic phenotypes for these major enzymatic activities (*e.g.*, high, intermediate and low); 3) identify variability in metabolic activity as a function of hormonal exposure and/or individual variability; and 4) determine the molecular mechanisms that account for any observed phenotypic variability. It is predicted that these studies will identify estrogen-specific enzyme isoforms expressed in the ovary that demonstrate significant hormonal responsiveness and/or interindividual variability. These studies have the potential to aid in the definition of a group of individuals at greater risk from estrogen exposures for developing hormonally induced cancers to have major implications for FDA monitoring of food-borne carcinogens.

In preliminary studies, the division has obtained evidence that the heterocyclic amine, 2-amino-α-carboline (AαC), 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 larynx. During 1998, they plan to complete characterization of this DNA adduct and develop an LC/MS method, in collaboration with the Division of Microbiology and Chemistry, to confirm adduct levels in human tissues. In addition, they have data that 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). 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 AαC and other smoking-related DNA adducts.

In collaborative studies in the area of molecular epidemiology, the division is planning human studies with the UAMS and the German Cancer Research Center 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 *GSTM1*. 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.

Because of their development of a rapid phenotyping assay for the phenol sulfotransferase (*SULT1A1*) in their colo-rectal case-control study (see 1997 Accomplishments), they will begin another collaborative case-control study with the UAMS and the NCI, funded through an IAG, on urinary bladder cancer susceptibility. Like colo-rectal 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.

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

In collaboration with the Division of Biochemical Toxicology at NCTR, ongoing studies involving the neonatal mouse bioassay (see 1997 Accomplishments) will provide data in 1998 on whether or not synthetic and natural estrogens and endocrine disruptors can serve as genotoxic carcinogens.

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.

9. International harmonization efforts on DNA adduct biomonitoring and its application to risk assessment.

In 1998, the division will continue to play a major role in international efforts on DNA adduct biomonitoring (see 1997 Accomplishments) and its use as a biomarker of exposure and disease risk.

10. Development and validation of DNA chip technology for human diagnostics.

In their continuing studies on individual differences in drug and carcinogen-metabolizing enzymes, they are in the initial stages of establishing CRADAs with companies that develop DNA chip technology for rapid, high-throughput genotyping. The goal is to be able to genotype patients for all the major variant enzymes that would enable them to predict adverse drug reactions and carcinogen susceptibility. They anticipate that such efforts could have a major impact on the ability to understand the likelihood of adverse drug reactions in susceptible subpopulations. 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.

FY 97 Accomplishments

During 1997, division studies on genetic polymorphisms were focused on the bioactivation and detoxification of the food-borne heterocyclic amines, which have been of increasing public health concern to FDA.

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; and a simple genotyping assay has been developed that is now being applied to 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 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 newly 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. They have recently discovered a genetic variant of *CYP1B1* and have developed a method for rapid genotyping of human populations. They have now found that this variant, in which isoleucine is replaced by valine in the protein, is significantly over-represented in patients with prostate cancer. A breast cancer case-control study is also underway. They expect 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. Recently, in collaboration with Professor Dong-xin Lin, a visiting scientist at NCTR and now Chief of the Molecular Epidemiology Division at the National Cancer Institute in Beijing, they have 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 food-borne 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 ORA/FDA.

Polymorphisms of phenol and estrogen sulfotransferases. 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 colo-rectal 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 colo-rectal and urinary bladder cancer.

In addition to the studies with human *SULT1A1*, the division has recently examined a newly identified human estrogen sulfotransferase (*SULT1E1*), that catalyzes the sulfonation of steroids, including endogenous and synthetic estrogens, and is present in the liver, breast, and endometrium. They have found that, of all the *SULTs* and NATs, only human *SULT1E1* sulfates α-hydroxy tamoxifen, a major metabolite of tamoxifen. This results in a highly reactive ester that forms the same DNA adducts reported to be present in human endometrium and thought to result in endometrial carcinogenesis. In a survey of 26 human livers, 50% of samples had no detectable *SULT1E1* activity. These data indicate that there is likely to be a mutant allele that is responsible for this lack of activity. Thus, individuals with the null genotype are hypothesized to have a higher risk of estrogen-related cancers, due to the lack of *SULT1E1* activity. Alternatively, breast cancer patients who are *SULT1E1*-positive would be expected to be at higher risk to tamoxifen-induced endometrial cancer. If this hypothesis can be confirmed by DNA sequencing, genotypic analysis should allow them to predict which women should be treated with tamoxifen and those for which alternate drugs should be considered.

Polymorphisms of GST A1, A2, and P1. In other studies on metabolic polymorphisms and cancer susceptibility, they have examined polymorphisms for the GSTs, specifically human GST A1, GST A2, and GST P1, which not only detoxify food-borne carcinogens, but are extremely active toward a wide variety of chemotherapeutic agents and are the most abundant drug detoxifying enzymes in most human tissues. Initial studies now show that both GSTs are polymorphically expressed in humans. These findings are of particular relevance since GST A1 is known to be inducible by consumption of fruits and vegetables, which is the most consistent protective factor against human cancers. GST P1, on the other hand, is involved in the conjugation of many chemotherapeutic agents, thereby decreasing their therapeutic efficacy. They have now examined three common alleles in GST P1 and developed genotyping methods that will allow them to test hypotheses regarding the ability to assess, on a individual basis, the likelihood of response to cancer chemotherapy by specific drug treatment regimens.

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 intra-strand 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, they have 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 roles of these enzymes in improving cancer chemotherapy.

Coffee and tea effects on *CYP1A2,* GSTs, and NATs. Since colo-rectal 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 was 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 A-class GSTs and down-regulation of NAT1 and NAT2 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. Using animal models in a collaborative study with the Division of Biochemical Toxicology, they have shown that calorie restriction markedly protected against changes in gene expression (through gene methylation) by maintaining *S*-adenosyl-methionine (SAM) levels and preventing the formation of pre-neoplastic lesions produced in the livers of rats treated with aflatoxin B_1 together with feeding a methyl-deficient diet. Similarly 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* and supports 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 formation and other pathologic effects even after a genetic alteration has occurred. Interestingly, they have 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 pre-neoplastic tissue constitutes one of the most ubiquitous occurrences in cancer. Another recent finding from this division was an abnormal methylating activity of DNA methyltransferase obtained from tumors developing in methyl-deficient rats; a similar enzymatic activity was not seen in normal

liver. This observation, if confirmed and extended, raises the prospect that the abnormal form of the enzyme may serve as an endogenous carcinogen.

In this regard, new findings from the division is expected to increase the validity of extrapolations between animal models and humans. The development by division staff of routine assays for SAM, *S*-adenosylhomocysteine (SAH), homocysteine, and 5-methyldeoxycytidine in blood should facilitate an evaluation of 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. In 1997, these findings were recognized by an Award Lecture on Nutrition and Cancer Prevention at the Annual Meetings of the AACR.

3. Etiology of human colo-rectal cancer: role of dietary heterocyclic amines.

In 1997, the division has continued its case-control study of colo-rectal cancer with UAMS and has enrolled about 100 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 food-borne carcinogens. Furthermore, with their development of a rapid phenotyping assay for *SULT1A1*, they have recently reported that the high activity phenotype is protective for colo-rectal cancer, with a 60% prevalence among controls and only 40% among cancer cases.

Thus far, this research has shown that, consistent with the proposed metabolic activation pathway for heterocyclic amine carcinogens, subjects at greatest risk for colo-rectal 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 food-borne heterocyclic amine carcinogen intake may account for over half of the sporadic colo-rectal tumor incidence observed in the U.S.

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

During the last year, extramural funding was received to support large case-control studies of breast and prostate cancer. In the last few months, recruiters who are breast cancer survivors have been trained and they have successfully enrolled over 30 women into the breast cancer study. A similar methodologic approach is being developed for the prostate cancer project.

5. Etiology of human pancreatic cancer: role of carcinogen & 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 150 cases and 400 controls entered into the study. In the interim, they have 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. The data indicate that environmental and endogenous adducts are present at comparable levels and appear to be due to smoking, airborne pollutants, and oxidative stress likely caused by dietary antioxidant insufficiency.

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 dideoxyinosine (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 them the ability to examine micronutrients 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.

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.

In its investigation of etiologic factors in breast and prostate cancer, the division has been striving to identify biomarkers of exposure. In the past year, exfoliated ductal epithelial cells have been separated from human breast milk, and DNA has been extracted. Aromatic carcinogen-DNA adducts were detected in these cells, and they are currently working to identify them. 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 food-borne 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 postnatal 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 effect (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.

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 evaluation 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 non-genotoxic processes have been proposed. However, in the neonatal mouse tumorigenicity bioassay, only two doses of the test compound, given to pre-weanling 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, they believe 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.

8. International efforts in molecular epidemiology, DNA adduct biomonitoring, and biotechnology.

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 WHO and several foreign governments and resulted in the publication of a 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.

International collaborative efforts in the area of human DNA adduct biomonitoring have also been ongoing in the division, together with the NCTR Division of Biochemical Toxicology, the U.S. EPA, and the International Agency for Research on Cancer (IARC). This working group has organized and is participating in interlaboratory trials for the detection of carcinogen-DNA adducts in humans and its application to human risk assessment. As part of NCTR's commitment to research progress in this area, the division served as a principal organizer and sponsor of the 1997 meeting of participants held at IARC (France). In 1998, the division will organize a similar meeting under the sponsorship of the American Association for Cancer Research. This effort now involves some 30 laboratories world-wide and is expected to form the basis for the use of DNA adduct measurements as biomarkers of exposure in making regulatory decisions by FDA, EPA, and the European Economic Community.

Finally, this division has played the lead role in organizing the newly formed Molecular Epidemiology Group of the American Association for 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.

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 subpopulations, 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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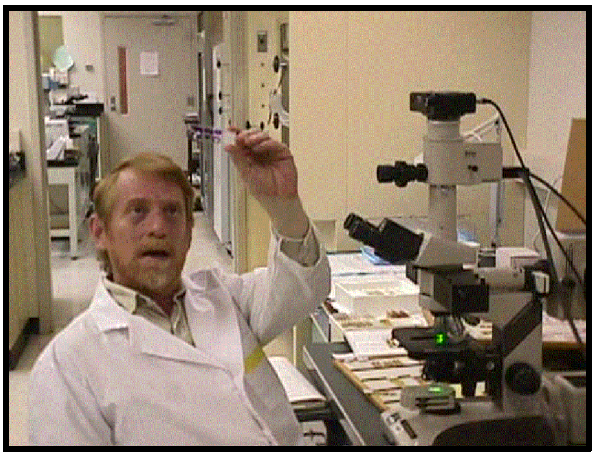

The utilization of a fluorescent stain, Fluoro-jade, as demonstrated by developer Dr. Larry Schmued, identifies dead or dying neurons on a slide from a domic acid exposed rat.

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.

FY 98 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 five 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 age and neuroanatomical susceptibility to neurotoxicants; 2) the role of aromatic monoamines in neurotoxicity; 3) disrupters of energy metabolism and axonal transport; 4) oxidative-stress-induced neurotoxicity; and 5) interspecies extrapolation and validation of animal models. Recently, a sixth focal area has been added entitled, neurohistochemical development and validation. 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 used to better understand developmental neurotoxicity and as an approach to elucidate the pathogenesis of neurotoxicants.

FY 97 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 enpoints in the developing monkey; to extend capabilities with NMDA receptor blockers and into the area of potential neuroprotective agents; and to utilize our 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.

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, 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 currently in progress 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 associated time course of histological effects.

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-tetrahydropynidine (MPTP) and will be utilized along with neurohistological methods and behavioral assessments of memory and learning. Reports have also been published on the oxidative-stress-producing potential of manganese and the importance of metal valence on neurotoxicity potency.

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 and hyper-kinetic 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 in this laboratory 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 attention deficit and hyperactivity disorder (ADHA). 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 Neurobiology/Neurotoxicology Working Group, ILSI Working Group on Human Variability, and "Red Book II" 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.

FY 98 Plans

Several research projects in the various focal research areas are scheduled for initiation in FY 98. Within the excitatory amino acid area, domoic acid-induced effects will be evaluated in the developing rat (collaboration with CFSAN). In addition, after the recent publication of a new sensitive and reliable fluorescent method for revealing neuronal degeneration and a simple, sensitive and reliable metallic stain for demonstrating myelin pathologies, a new research focal area on neurohistological technique validation has been initiated. In the monoamine focal area, the influence of body temperature on d-fenfluramine induced neurotoxicity will be further explored and studies on this and other stimulants (*e.g.*, methylphenidate and ephedrine) will be completed (collaboration with CDER).

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). Also, molecular methods for detecting brain cell type switching will be published. 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. 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.

New areas of effort within the neurotoxicology research group follow along the lines of the NCTR Science Advisory Board (SAB) Neurotoxicology Subcommittee recommendations and include: 1) the development of a neurotoxicology cell culture facility to investigate the neurotoxic potential of the anti-HIV therapeutics, fumonisins (CFSAN collaboration) and other FDA-relevant agents; 2) the development of molecular biology techniques to detect and describe the effects of aromatic monoamines (*e.g.*, methamphetamine, fenfluramine, methylphenidate) on neurotrophic factors

and agents postulated to induce oxidative stress (amphetamines, metals and MPTP); and 3) the completion of the electrophysiology laboratory to continue the studies on 3-NPA and domoic acid neurotoxicity and aid in the risk assessment of these agents.

Progress has been achieved in the study on the 3-nitropropionic acid (3-NPA)-induced chemical hypoxia. A recently published manuscript contains data on the increasing concentrations of the free fatty acids following 3-NPA that supports the hypothesis of oxidative stress underlying 3-NPA neurotoxicity. The 3-NPA protocol contributes not only to understanding the potential human neurotoxicity of this important mycotoxin and food contaminant but also to the further development of state-of-the-art electrophysiological methods in the division's Neurophysiology Laboratory. The development of this laboratory is an important addition to the division's capabilities as it clearly enhances their ability to further define the actions of neurotoxicants.

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.

In addition, the proceedings of the “Third International Conference on Neuroprotective Agents,” and the Satellite Conference on Mechanisms of Drugs of Abuse (supported and organized by members of the Division of Neurotoxicology) and consensus-building documents concerning the neurotoxicity assessment of food contaminants and other FDA-regulated agents (CFSAN collaboration) will be published.

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 [FB_1], 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. 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; and the “S” number indicates a research support project.

Principal/Co-Principal **Investigator**. The Principal Investigator (PI) is identified in bold type for each project. If the PI is not an NCTR employee, the PI's affiliation is printed as a footnote. For a complete listing of principal investigators, see Index, "Principal Investigator by Project."

Status/**Res. Area**/GOAL. Status indicates if the project was "Active" or "In Review" at the end of fiscal year 97, "Completed" during FY 97 or "Proposed" for FY 98. The Res. Area (research area) indicates the abbreviation for the NCTR research area responsible for the conduct of the project. A listing of the full name of the research area and its abbreviation is listed in the Table of Contents. **NOTE:** Two divisions have categorized their research into two laboratories which are indicated under Res. Area, i.e., the Division of Genetic (Gen Lab) and Reproductive Toxicology (Repro Lab) and the Division of Microbiology (Micro Lab) and Chemistry (Chem Lab). The abbreviation of the related NCTR strategic goal is listed in all capitals. A description of these goals can be found in the Preface on page iii.

Title. In parenthesis following the "Title" is the acronym for the collaborating FDA Center, if applicable. 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)
Office of Women’s Health (OWH)

RESEARCH PROJECTS

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0026200	**Cerniglia**	Active/ **Micro Lab/** METH	Microbiological Diagnostic Methods: Development, Testing, & Evaluation	To improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology and serology as applicable to NCTR programs and projects.
E0211201	**Howard**	Active/ **Bio Tox**/ AGNT	Tumor Promotion by Fumonisin B_1 in Male F344 Rats	Determine if fumonisin B_1 is a tumor promotor, using a classical initiation/ promotor design [administration of fumonisin B_1 one week after three weeks administration of methylbenzylnitrosamine]. Determine if coadministration of fumonisin B_1 with initiator methylbenzylnitrosamine results in an altered tumor yield as compared to tumor promotion study.
E0260401 E0260412	**Chou** Chung Fu	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 B1 (AFB1), benzo(a)pyrene (BaP), and 4-aminoazobenzene (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-enzymatically; 4. change activities of mouse-liver zenobiotic metabolizing enzymes, especially the hepatic glutatione 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
E0637100 E0637102	**Leakey** Harmon	Completed/ **Chem Lab/** CNPT	Profiles-Hepatic Detoxicating Enzymes	1. To complete the collection and analysis of human post mortem liver. 2. Study development of human and animal drug metabolizing enzyme proteins by using immunoblotting techniques.
E0657300 E0657301 E0657302 E0657303 E0657304 E0657305 E0657306 E0657307 E0657308 E0657312 E0657318	**Fu** Dooley Herreno-Saenz Kadlubar Von Tungeln	Active/ **Bio Tox/** PRED	Tumorigenicity of Nitro-Polycyclic Aromatic Hydrocarbons (Nitro-PAHs) and their Metabolites in the Neonatal B6C3F1 Mouse	1. To determine the tumorigenicity in the neonatal B6C3F1 mouse of a series of parent nitro-PAHs, and their ring-oxidized and nitro-reduced metabolites, that have been found to be mutagenic in the *Salmonella typhimurium* strains. 2. To determine structure-activity relationships of nitro-PAHs, as well as to determine the structural features that can affect tumorigenicity. 3. To determine if bacterial mutagenicity correlates with tumorigenicity of the nitro-PAHs selected for study. 4. To 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** Vaughn	Active/ **Biometry/** 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 Casciano Schol	Active/ **Gen Lab/** METH	Reliable Methodology for Cryopreservation	1. To develop a reliable methodology for cryopreservation of isolated hepatocytes. 2. To 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.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0663811 E0663813 E0663814 E0663815 E0663816	**Ferguson** Holson Ali Hansen LaBorde Webb Paule	Completed/ **Neuro Tox/** AGNT	The Effect of Neonatal Dexamethasone (DEX) Exposure on Brain and Behavior in the Rat	1. To determine whether neonatal exposure to DEX reduces neonatal regional brain growth. 2. To determine whether neonatal DEX effects on brain growth resemble those caused by the antimitotic compound methylazoxymethanol (MAM). 3. To determine whether neonatal effects on brain growth persist into early adulthood. 4. If DEX effects are seen on neonatal brain growth, to determine whether these effects are reflected in behavior, and whether such behavioral effects resemble those caused by neonatal MAM exposure.
E0665900 E0665901 E0665921	**Lu** Hart Turturro	Active/ **Repro Lab/** CNPT	Effects of Dietary Restriction on Cell Cycle Analysis in Rats and Mice	Study and compare the difference in cell proliferation between non-carcinogen and carcinogen treated animals, both ad libitum and CR restricted groups.
E0666900	**Fu** Von Tungeln	Active/ **Bio Tox/** CNPT	Comparative Regioselective and Stereoselective Metabolism of 7-Chlorobenz[a]anthracene and 7-Bromobenz[a]anthracene by Mouse and Liver Microsomes	To 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 E0667701	**Poirier** Lyn-Cook Zapisek	Active/ **Mol Epi/** CNPT	A Study to Determine if the Carcinogenic Effect of a "Methyl-Deficient" Diet on Rats Can Be Reversed by a "MethylSufficient" Diet	Study methylation pattern of liver DNA and liver carcinogenesis in rats on methyl deficient diet then switched to methyl sufficient diet.
E0671700	**Pipkin** Hinson Lyn-Cook	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).

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0671900 E0671911 E0671921 E0671931	**Ali** Bowyer Carrington Holson Lipe Melethil Newport Scallet Siitonen Slikker Sobotka Soliman	Active/ **Neuro Tox/** AGNT	Neurotoxicity Assessment of Prenatal, Postnatal, and Adult Exposure to Manganese in the Rat (CFSAN)	1. To determine whether administration of Mn during prenatal, postnatal and adult periods produces: i. any significant accumulation of Mn in plasma and different regions of the rat brain, ii. alterations in the dopaminergic neurotransmitter system, as evidence by a) changes in concentration of dopamine and its metabolites; b) in dopamine receptor binding and dopamine release, and c) in the rate limiting enzyme tyrosine hydroxylase. 2. To determine whether accumulation of Mn in the divalent (Mn+2) or trivalent (Mn+3) state is associated with producing neurotoxicity. 3. To determine if Mn accumulation and neurotoxicity is enhanced if administered to iron deficient rats during prenatal, postnatal or adult periods.
E0672900	**Thompson** Caper Siitonen	Active/ **Chem Lab/** METH	Development of Method(s) for Analysis of Cadmium and Lead in Calcium Supplements by Graphite Furnace Atomic Absorption Spectrophotometry (CFSAN)	1. Develop and validate a furnace AAS method for analysis of Cd and Pb in Ca supplements. 2. Analyze a sufficient number of different Ca supplements obtained from local health food businesses to determine applicability of method to different Ca matrices.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0676600	**Leakey** Casciano Domon Frame Harmon Lee McGarrity Morris Shaddock Zielinski	Completed/ **Chem Lab**/ AGNT	*In Vitro* Metabolism of Topical Antimicrobials (CDER)	1. To determine the *in vitro* metabolic profiles of the topical antimicrobial, chlorhexidine digluconate (CHD) and p-chloro-m-xylenol (PCMX), in rat, human, and rhesus monkey liver preparations and in human lymphoblastoid cells transfected with individual drug metabolizing isozymes. 2. To assess the potential mutagenicity of the major metabolites of CHD and PCMX in human lympho-blastoid AHH-1 TK+/- cells, trans-fected with individual human cytochrome p450 isozymes. 3. To assess the validity of *in vitro* enzyme assays for predicting the metabolic fate of chemicals *in vivo*.
E0676700 E0676711	**Aidoo** Casciano Lyn-Cook	Active/ **Gen Lab** PRED	Development of an Assay to Measure 6-Thioguanine-Resistant Rat T-Lymphocytes Treated with Mutagenic Agents *in vitro*	To develop techniques for *in vitro* mutagenicity studies with rat lymphocytes.
E0676900	**Lu** Hart Zhang	Active/ **Repro Lab**/ CNPT	DNA Repair and Cellular Responses of Dietary Restricted Rats Exposed to Carcinogens	To examine DNA repair in glandular stomach & brain tissues of ad libitum fed male rats exposed to potent carcinogen methyl-N1-nitro-N-nitrosoguani-dine (MNNG) or methyl nitro-sourea (MNU), respectively. To develop and establish bromodeoxyuridine incorporation to study cell proliferation response in glandular stomach tissue of dietary restricted rats.
E0677000	**Rowland** Hinson Lyle Swicord	Active/ **Bio Tox/** AGNT	Investigation of Neoplastic Transformation Induced *In Vitro* by 60 Hz Magnetic Fields (CDRH)	Determine if 60 Hz sinusoidally varying continuous wave magnetic fields can induce or promote neoplastic transformation *in vitro*.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0677500	**Delclos** Blaydes Heflich Jacobson Smith	Active/ **Bio Tox/** AGNT	DNA Damage in Mammary Tissue, Liver and Nucleated Blood Cells of F344 Rats with Polyester Polyurethane (Microthane Foam) Implants (CDRH)	To develop methods for the detection of low levels of DNA damage produced by metabolites of 2,4- and 2,6-toluenediamine.
E0678100	**Casciano** Domon McGarrity Morris Sotomayer	Active/ **Gen Lab**/ PRED	Metabolism and Induction of Mutations by the Fermented Food-Borne Carcinogen Urethane in the Transgenic Human Lymphoblastoid Cell H2D6 (CFSAN)	To isolate and quantify spontaneous and urethane-induced mutations at the hypoxanthine guanine phosphoribosyl transferase (hprt) and the thymidine kinase (tk) loci in transgenic human lymphoblastoid cells expressing CYP2E1; to determine if urethane induces primarily single gene mutations or multilocus mutations; and to describe conditions which modulate metabolic activation of urethane to genotoxic metabolites.
E0678500	**Lay** Chiarelli Gay Sphon	Completed/ **Chem Lab/** METH	Development of FAB/MS and FAB/MS/MS Methodologies for the Analysis of Peptides (CFSAN)	To develop, using the combined resources available at CFSAN and NCTR, a capability for the analysis of peptides up to 3500 daltons via FAB/MS and FAB/MS/MS.
E0678600	**Cerniglia** Wang	Active/ **Micro Lab/** PRED	Microbial Studies on Macronutrient Food Substitutes Phase I Validation Studies (CFSAN)	1. To validate the semicontinuous culture system for further studies on effect of macronutrient food substituents on the microbial activity and ecology of human intestinal micro-flora.
E0678800 E0678801 E0678820	**Feuers** Berg Duffy	Active/ **Gen Lab/** AGNT	Acute Toxicity of Ganciclovir: Circadian Response and Effect of Dietary Restriction	1. Determine if a circadian effect on the acute toxicity of ganciclovir can be demonstrated. 2. Determine if CR has an impact on the chrontoxicology of ganciclovir.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0679500	**Casciano** Bradlaw Domon McGarrity Morris Page Shaddock	Active/ **Gen Lab/** PRED	Evaluation of Cytotoxic Properties of Chemical Components Associated with L-Tryptophan Contamination Using Transgenic Human Lymphoblastoid Cell Lines (CFSAN)	To identify biologically active contaminants of L-tryptophan through the use of cytotoxic indices in transgenic human lymphoblastoid cells.
E0680500	**Casciano** Taylor Wamer	Active/ **Gen Lab/** CNPT	Does β-carotene Modulate the Effects of Carcinogens on Gene Expression (CFSAN)	Determine if β-carotene can modify the carcinogen induced expression of various oncogenes and tumor antigens in SV40 transformed CHO cells.
E0681300	**Shuttleworth** Cerniglia Davis Luneau	Completed/ **Micro Lab/** CNPT	Investigations of the Content and Fate of PAHs in Sediments Collected Near Commercial Fishing Areas and the Effects of Bioaugmentation with a PAH Degrading *Mycobacterium Sp.* in Environmental Microcosms	1. To determine the usefulness of a *Mycobacterium sp.* in the removal of organic pollutants from contaminated sediments. 2. To test the ability of this *Mycobacterium sp.* to mineralize PAHs in heavily contaminated sediments.
E0681600	**Aidoo** Lyn-Cook Wamer	Active/ **Gen Lab/** CNPT	Studies on Antioxidants: Evaluation of the Mutagenic Activity on N-Ethyl-N-nitrosourea (ENU) in the Rat (CFSAN)	1. To pre-treat F344 rats with anti-oxidant vitamins: β-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. To 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.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
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-tetrahydropy-nidine (MPTP) Administration on Complex Brain Function, Neurochemistry and Neuro-histology in the Rhesus Monkey (CFSAN)	Chronic administration of low doses of the dopaminergic neuro-toxicant MPTP will lead to detect-able 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 Carcino-genicity Study of Iron Overload (CFSAN)	To determine the dose levels of carbonyl iron to be used in a subsequent chronic bioassay.
E0682900	**Lipe** Ali Carrington Newport Slikker	Active/ **Neuro Tox/** AGNT	Effect of Manganese on the Concentration of Amino Acids in Various Regions of the Rat Brain (CFSAN)	1. To determine if exposure to manganese alters amino acid concentrations in selected regions of the adult rat brain. 2. To determine if exposure to manganese alters amino acid concentrations in selected regions of the developing rat brain.
E0683400 E0683401 E0683402	**Feuers** Deluca York	Active/ **Gen Lab/** CNPT	The Effect of Age and Food Restriction on Glutathione S-Transferase Isozymes in C57BL/6N Mice	This study seeks correlations between aging, caloric restriction, and BHA administration with alterations in GSTase isozyme composition.
E0683700	**Paule** Gillam Slikker	Active/ **Neuro Tox/** AGNT	Effects of Chronic Methly-phenidate (Ritalin) Administration on 'Cognitive' Functions in the Rhesus Monkey	To determine whether chronic treatment with relevant doses of the therapeutic agent methyl-phenidate (Ritalin) will result in detectable changes in specific 'cognitive' abilities in a nonhuman primate model of complex brain function.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0684000	**Colvert** Ferreira Holland Rafii	Active/ **Micro Lab/** METH	Detection of *Clostridium botulinum* Using Enzyme Linked Immunosorbent Assay and Polymerase Chain Reaction Techniques (ORA)	The primary objective is to develop better *in vitro* methods for the detection of *C. botulinum*.
E0684400	**Colvert** Holland Noah	Completed/ **Micro Lab/** METH	Production of Monoclonal Antibodies Against *Vibrio cholerae* for Diagnostic Screening Tests: Establishment of Monoclonal Antibody Capabilities at NCTR (ORA)	A rapid method is needed to screen food samples for *Vibrio cholerae* at the FDA.
E0684500	**Paule** Clausing Appel	Completed/ **Neuro Tox/** AGNT	Preliminary Assessment of a Method for Screening Potential Neurotoxic Effects of Prenatal Alcohol Exposure using Autoradiographic Measurement of Cellular Metabolic Markers (CDER)	1. To examine, validate and compare the utility of a number of biochemical markers of neurotoxic insult that can be detected using autoradiographic methods.
E0684800	**Littlefield** Hass Poirier	Active/ **Bio Tox/** AGNT	The Effect of Dietary Magnesium on the Induction of Tumors, Transformation of Cells, and Leukemia Incidence	1. To 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 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	Determine if mutations at the hprt locus of lymphocytes from Sprague-Dawley rats treated with DMBA can be used as a biomarker for the induction of mammary tumors.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0685300	**Yerokun** Heflich	Active/ **Gen Lab/** PRED	Construction of Transgenic Hamster Ovary Cells Expressing Arylsulfotransferases IV and Their Use in Studies of Molecular Mechanisms of Arylamine- and Polycyclic Aromatic Hydrocarbon-Induced Carcino-genesis	1. To construct a mammalian expression vector containing the AST IV gene and to transfect CHO cells with the recombinant vector, and 2. to use these transgenic cells in the hypoxanthine- guanine phos-phoribosyl transferase (*hprt*) and adenosine phosphoribosyl trans-ferase (*aprt*) mutation assays.
E0685800	**Jackson** James	Completed/ **Repro Lab/** CNPT	Requirements for Dietary Nucleotides and Folic Acid in Rapidly Dividing Cells: II. Effect of Partial Hepatectomy on Foci and Tumor Development	Determine if purified and semi-purified diets such as AIN-76A that lack preformed nucleotides will compromise DNA synthesis in tissues undergoing rapid cell proliferation and if this interference in DNA synthesis is associated w/alterations in deoxynucleo-tide pools & a greater risk for cell transformation.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0685900 E0685911	**West** Beland Delclos Fu Rowland	Active/ **Bio Tox/** CNPT	The Transformation Potential of Nitro-Polycyclic Aromatic Hydrocarbons Assayed by the Rat Trachael Epithelial Cell (RTE) Systems	To optimize conditions for the transformation of RTE cells *in vitro* with nitro-PAHs. To characterize the metabolic capability of the primary RTE cell population using 1,6-dinitropyrene & 6-nitro-chyrysene as substrates. To characterize the DNA-adduct profile in primary RTE cells after exposure to 1,6-dinitropyrene & 6-nitrochry-sene. To determine the transformation potential of 1-nitrosopy-rene, 2-nitropyrene, and 1-,2-, & 3-nitrochrysene, so as to extend the data base for evaluation of SARs w/nitro derivatives of pyrene & chrysene already begun under E-6742. To determine the trans-formation potential of certain nitro derivatives of benzo(a)pyrene and benzo(e)pyrene.
E0686501	**Hass** Chen Littlefield	Active/ **Repro Lab/** AGNT	Verification of Methapyrilene, a Human Antihistamine and a Rat Liver Carcinogen, as an Agent that Alters Phenotypic Expression of Transformed Cells	This study is the first phase of several possible sequential studies designed to investigate the potential uniqueness of MP as an anti-tumor agent. The objective of this study is to establish the hypothesis that MP at certain non-toxic concentrations is an anti-transformation agent.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0686701 E0686711 E0686721	**Hansen** Dial Grafton	Completed/ **Repro Lab/** PRED	Investigations on the Mechanism of Valproic Acid (VPA)-Induced Embryotoxicity *In Vitro*	To 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, ormate, 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*.
E0686800	**Freni**	Active/ **Biometry/** AGNT	Fluoride in Public Drinking Water Systems and Human Fetal Health	Investigate the statistical association of exposure to fluoride in drinking water and fetal death, prematurity, low birth weight, and infant death in the counties and states in the U.S. included in project E-6733.
E0687001	**Ahn** Kodell	Active/ **Biometry/** METH	Nonparametric Estimation and Testing of the Tumor Incidence Rate in Survival/Sacrifice Experiments	To 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 Lab/** METH	Development of Devices/Methods for Determination of Food/ Seafood Quality (ORA)	Assist FDA with problems incurred in testing seafood for decomposition by developing an expeditious assay for determining volatile and semivolatile organic compounds in spoiled seafood.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0687501 E0687511 E0687521 E0687531 E0687541	**James** Poirier Wise	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.
E0687701	**Wang** Cao Cerniglia	Completed/ **Micro Lab/** METH	Development of Species-Specific DNA Probes and PCR Methods for Rapid Detection of Anaerobic bacteria: *Clostridium perfringens, C. clostridiiforme, C. leptum, Bacteroides distasonic, B. vulgatus, B. thetaiotaomicron*	Develop simple, specific, sensitive and reliable methods for rapid identification and *detection of anaerobic bacteria: Clostridium perfringens, C. leptum, C. clostridiiforme Bacteroides distasonis, B. vulgatus, B. thetaiotaomicron* and other related species.
E0687801 E0687811	**Lyn-Cook** 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. To determine the effects of the antioxidant vitamins on the genotoxicity induced by exposing mutagens/carcinogens to young and old rats. 2. To determine the effects of antioxidant vitamins on lymphocyte function in mutagen-exposed and non-exposed young and old rats.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0687901 E0687912 E0687913 E0687914 E0687915 E0687916 E0687917	**Fu** Casciano Contrera Kadlubar Teitel VonTungeln Doerge Poirier	Active/ **Gen Lab/** PRED	The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lym-phoblastoid Cells (CDER)	1. To determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs. 2. To examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo(a)-pyrene, 6-nitrochrysene, & aflatoxin B1 3. To study the metabolism and DNA adduct formation of benzodiazepine and antihistamines drugs by mouse and human liver microsomes to determine which, if any, cyto-chrome 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** Dial Grafton	Completed/ **Repro Lab/** CNPT	Investigations on Carbama-zepine (CBZ) Embryotoxicity *In Vitro*	1. To determine if exposure of embryos *in vitro* to carbamaze-pine (CBZ) alters normal growth and development. 2. To determine if a stable epoxide metabolite of CBZ or metabolic activation of CBZ by microsomes alters normal growth and development of embryos. 3. To determine if any of three folate derivatives will ameliorate potential embryotoxicity due to exposure *in vitro* to 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 Antidepressant Fluoxetine (CDER)	1. To determine if chronic feeding of fluoxetine (Prozac) results in promotion of mouse mammary carcinomas. 2. To 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 Deorge Fishman Medlock Sheehan Streck	Active/ **Repro Lab/** KNLG	Effects of Therapeutic Antiestrogens on Postnatal Uterine Development in the Rat (CDER)	To 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. To assess uterine estrogen receptor modulation by neonatal antiestrogen exposure.
E0688601	**Fishman** Branham Sheehan Streck	Active/ **Repro Lab/** KNLG	In Situ Expression of Estrogen Receptor (ER) Protein and mRNA in the Developing Reproductive Tract	To analyze estrogen effects on ER levels in the developing reproductive tract at the cellular and molecular genetic level.

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E0688701 E0688711	**Ali** Cadet Freyaldenhoven Newport Slikker	Active/ **Neuro Tox/** CNPT	Evaluation of Constitutive and Stress-Induced Levels of Expression of Heat-Shock Proteins (HSP) in Cu/Zn-Superoxide Dismutase Transgenic Mice	1. Determine whether there are significant differences in constitutive HSP expression in Cu/Zn-Superoxide Dismutase (SOD)-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 MPTP in 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 litter-mate 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 E0688811	**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. To 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. To study correlations between the chemically-induced tumor incidence and various biomarkers of the initiation and the promotion stage of carcinogenesis for continuous and intermittent dosing. 3. To determine whether nutritional status can alter the sensitivity to carcinogen dose, the expression of various biomarkers, and cancer risk assessment.
E0688901	**Shaddock** Feuers	Active/ **Gen Lab/** METH	Effect of Cryopreservation and Long-Term Storage of Primary Rodent Hepatocytes on 125 I-Insulin Uptake and Binding and the Regulation of Hepatic Pyruvate Kinase by Insulin and Glucagon Treatment	1. The primary objective of this study will be to measure Km and Vmax of hepatic pyruvate kinase for phosphoenol pyruvate in freshly isolated and cryopreserved rodent hepatocytes. 2. Evaluate the ability of insulin to dephosphorylate and glucagon to phosphorylate hepatic pyruvate kinase in freshly isolated and cryopreserved rodent hepatocytes. 3. Evaluate the ability of insulin to stimulate hepatic pyruvate kinase synthesis and glucagon to decrease the levels of pyruvate kinase synthesis in freshly isolated and cyropreserved rodent hepatocytes 4. Measure 125 I-Insulin uptake and binding in freshly isolated and cryopreserved rodent hepatocytes.

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E0689001 E0689011	**Streck** Rajaratnam Fishman Streck 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	To determine whether experimentally inducing 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 normogly-cemia 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	**Medlock** Burroughs Faber Hughes Sheehan Whitten	Active/ **Repro Lab/** KNLG	Alterations in Reproductive Tract Morphology and Biochemistry in Rats Treated Neonatally with Phytoestrogens	1. To determine if the 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 DES. 2. To determine if the phytoestrogens, when given neonatally cause the same morphological alterations in the female reproductive tract at 6 and 10 months as DES. 3. To determine if the 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** Kadlubar Lin	Active/ **Mol Epi/** PRED	Chemoprotection of DNA Adducts of 2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine in the Rat	To examine the effect of the GSH S-transferase inducers, phenethylisothiocyanate, diallyl sulfide (DAS), 5-(2-pyrazinyl)4methyl1,2-dithiol 3-thione (Oltipraz), garlic powder, cabbage powder, 2(3)-tert-butyl-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	To 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. To develop a method for inputing the number of fatal tumors in an experiment that lacks cause-of-death data, in order to modify the IARC cause-of-death test. To develop a procedure for estimating the lag time between onset of and death from an occult tumor, when cause-of-death data are unavailable. To illustrate the new procedures using data from the PCR studies.

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E0689801 E0689813	**James** Basnakian Gaylor Hart Kammula Mishra Muschelishvili Pogribny Stratmeyer Turturro	Completed/ **Bio Tox/** CNPT	Mechanisms of Foreign-Body Carcinogenesis: The Role of Inflammation, Proliferation, and Cell Death (CDRH)	1. At the descriptive level: to determine with *in situ* immunohistochemical techniques, a) the early infiltration of CD4+, CD8+ T lymphocytes, B cells and inflammatory leukocytes into the area immediately adjacent to implant material... 2. At the mechanistic level: determine a) whether rates of proliferation and apoptotic cell death are altered in cells adjacent to implant material. 3. At the prognastic level: define the "survival index" in the early stages of foreign-body carcinogenic process that would be predictive of the subsequent development of dysplastic tissue and/or local sarcoma.
E0690001	**Khan** Cerniglia Eirkson Jones	Completed/ **Micro Lab/** METH	Development of a Detection Method for Tracking Genetically Engineered Microorganisms using Polymerase Chain Reaction and DNA-DNA Hybridization Methods (CVM)	To develop a rapid and sensitive detection method for tracking genetically engineered microorganisms (GEMS) in environmental microcosms.
E0690101	**Pothuluri** Assaf Cerniglia Nawaz	Active/ **Micro Lab/** METH	Microbial Degradation of Drugs and Feed Additives Used in Fish Farming (Aquaculture) (CVM)	To develop a standardized method to evaluate the biodegradation of drugs and feed additives used in fish farming (aquaculture). To determine the biodegradation rates and metabolic fate of the antibiotic erythromycin in aquaculture water and sediments.
E0690201	**Kodell** Chen Lin	Active/ **Biometry/** PRED	Bioassays of Shortened Duration for Drugs: Statistical Implications (CDER)	To 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	Active/ **Neuro Tox/** CNPT	Factors Affecting the Neurotoxicity of Amphetamines and Related Compounds	1. To determine how age, mode of administration, and environmental temperature during drug exposure alter the neurotoxicity of fenfluramine and methylpheni-date. 2. Measure the effects of age and environmental temperature on the pharmaco-kinetics of several of the amphet-amines. 3. The effect of neurotoxic doses of 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 d-fenfluramine neuro-toxicity 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 continous nonacute 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	Active/ **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 LaBorde Paule	Active/ **Neuro Tox/** AGNT	Neural and Functional Teratogenesis of Retinoids in the Rat	1. To 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. To identify and characterize the age-specific functional and neurological alterations produced by the above doses. 3. To assess within-animal and within-litter correlations between functional and underlying neurological abnormalities induced by retinoids.

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E0690601 E0690611	**Manjanatha** Aidoo Casciano Heflich Lyn-Cook Mittelstaedt Shelton	Active/ **Gen Lab/** PRED	Quantitative and Molecular Analysis of 7,12-Dimethylbenz-[a]anthracene (DMBA)-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. To determine the mutant frequency and mutation spectrum of the lacI 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. To 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. To 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.
E0690801	**Zheng** Kodell	Active/ **Biometry/** METH	Properties of the Hazard and Survival Functions of the Moolgavkar, Vinzon, Knudson (MVK) Stochastic Carcinogenesis Model	Investigate mathematical properties of the 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.

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E0690901	**Chen** Ahn Tsong	Completed/ **Biometry/** METH	A Linear Mixed Effects Model for Analysis of Data for Stability Studies (CDER)	1. To investigate the methods proposed in the literature for improvement of the current FDA recommended procedure. 2. To investigate and develop a linear mixed effects model for statistical analysis of stability of a drug or biological product. 3. To develop a procedure for estimating a confidence limit on the predicted response for determining expiration period of a drug or biological product. 4. To investigate and develop computational procedures for estimating the regression coefficients of the fixed effects, mixed effects, and random effects, and variance components. 5. To investigate and develop procedures for testing the equality of variances of different batches. 6. To investigate and develop procedures for testing the equality of regression coefficients when the batch variances are unequal. 7. To compare the linear mixed effects approach with the current procedure recommended by the FDA.
E0691001	**Gaylor** Chen	Active/ **Biometry/** PRED	Upper Limit for the Sum of the Risks of the Components in a Mixture and an Optimum Strategy for Risk Reduction	To 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.

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E0691201 E0691211 E0691221	**Wolff** Ali Whittaker	Active/ **Bio Tox/** AGNT	Cellular and Molecular Responses to Chronic Iron Overload in Animal Models (CFSAN)	1. To determine the health effects of chronic iron overload in mice and rats. 2. To determine neurochemical changes after chronic iron overload in mice and rats. 3. To 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's and Alzheimer's diseases.
E0691401 E0691411 E0691421	**Frederick** Fogle Paule	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 Neurobehavio-ral Toxicity	1. To 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. To determine the relative sensitivities of the behavioral endpoints monitored in the rodent OTB to pharmacological disruption. 3. To 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. To validate the use of rodent operant performance as useful predictors of neurobehavioral toxicity. 5. To add to existing knowledge of the neurochemical and neurophysiological basis of complex brain functions.

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E0691501	**Hansen** Pauken Sonneborn Terry	Active/ **Repro Lab/** PRED	Stress Protein Expression Following Treatment with Developmental Toxicants *In Vitro*	To 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; to determine the kinetics of stress protein mRNA syntheses; to determine if this mRNA is translated into newly synthesized stress proteins; and to determine location of stress proteins in treated embryos by immunohistochemical detection.
E0691801	**Terry** Hansen	Active/ **Repro Lab/** PRED	Immunohistochemical Localization of Folate in the Neural Tube at the Time of Closure	To 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; to 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; and to 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.

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E0691901	**Ahn** Barton Chen Hertzberg Kodell Springer	Completed/ **Biometry/** METH	Simulation Study on Reducing Conservatism in Risk Estimation for Mixtures of Carcinogens (CFSAN)	To conduct a simulation study of methods for estimating upper bounds on excess cancer risk from exposure to a mixture of carcinogens, under the assumption of low-dose additivity of risks; to compare the common procedure of simply summing individual upper bounds on risk to less conservative estimating procedures, in order to investigate the reduction of conservatism in the total risk estimate; to determine if the reduction in conservatism achieved by the less conservative procedures makes it worthwhile for regulatory agencies to change from the common practice of summing upper bound risk estimates.
E0692001 E0692011	**Doerge** Divi Chang Churchwell Holder	Active/ **Chem Lab/** CNPT	Toxic Hazards from Anti-Thyroid Chemicals	Determine inhibition mechanisms for environmental goitrogens using purified thyroid peroxidase and lactoperoxidase; Determine the mechanism for covalent binding suicide substrates to purified peroxidases using electrospray mass spectrometry to analyze intact adducted proteins and/or proteolytic fragments; Determine mechanism of goitrogen uptake into isolated thyroid cells in primary culture and subsequent inhibition of iodination/ coupling reactions involved in thyroid hormone synthesis; Determine the structure-activity relationship for uptake of goitrogens into the thyroid and inhibition of thyroid hormone synthesis rats.

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E0692201	**Sutherland** Cerniglia Eppley Freeman Wilkes	Active/ **Micro Lab/** AGNT	Microbial Metabolism of Fumonisin (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 for use in mammalian studies.
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. To evaluate the effects of the developmental neurotoxin 3-NPA on NMDA, dopaminergic and serotonergic systems using neurochemical methods. 2. To evaluate the neurohistological effects of calcium-mediated vs. serum-mediated stimuli on the expression of stress proteins (c-fos). 3. To correlate 3-NPA toxicity with age.

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E0692401 E0692411 E0692421	**Duffy** Allaben Chanderbhan Feuers Hart Hass Hattan Leakey Lewis Lyn-Cook Pipkin Tang Taylor Turturro	Active/ **Repro Lab/** CNPT	Effect of Different Levels of Caloric Restriction on Physiological, Metabolic, Biochemical, Immunological, Molecular, and Body Composition Variables in Rats (CFSAN)	To determine how various levels and durations of CR affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, etc; 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; 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; 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; 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; Determine temporal and environmental factors that modulate the toxicity of foods, food additives, and food substitutes; Develop experimental methods for utilizing CR in the chronic bioassay; Develop control data for a reference purified diet that has been formulated to conform to long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies; Provide control data for the calculation of risk and levels of safety related to food and drug toxicity.

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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** Frame Lyn-Cook Slikker Stewart Tank	Active/ **Neuro Tox/** METH	Implementation of Molecular Biological Techniques for Assessing Changes in Neuro-growth/Neurotrophic Factors after Exposures to Neurotoxic-ants and Other Substances	Select and produce/obtain cDNA and RNA probes for detecting changes in message RNA (mRNA) levels for the various neurogrowth/neurotrophic (NTFs) which are likely to be involved in either secondary mechanisms of neurotoxicity or repair after neuro-toxicant insult. Detect changes in NTF mRNAs after insult to neuro-toxicants and other substances, and determine if these are the same for 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 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.
E0692801	**Evans** Komoroski Mrak	In Review/ **Chem Lab/** CNPT	Metabolite Changes in Alzheimer Disease (AD) by ^{1}H NMR	Using high resolution of ^{1}H NMR of methanol/water extractors, we will compare the metabolite profiles in four key regions of the brain for 36 patients, definitively diagnosed with AD by postmortem neuropathologic assessment, with those of 24 controls, matched for age and postmortem interval. Within the AD group, we will correlate the concentrations of the various metabolites with the severity of the disease.

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E0692901	**Terry** Hansen Streck	Active/ **Repro Lab/** PRED	Toxicant Effects on Neural Cell Adhesion Molecule and N-Cadherin during Mouse Neural Tube Closure	To determine the optimum time of neural cell adhesion molecule (NCAM) and N-Cadherin expression in the closing CD-1 mouse neural tube; to quantitate changes in neural fold NCAM and N-cadherin levels following embryonic exposure to valproic acid, lithium or heat *in vivo*.
E0693001	**Scallet** Ali Hall Johannessen Paule Rountree Sandberg Schmued Slikker Sobotka	Active/ **Neuro Tox/** AGNT	Estimating Quantitative Neurotoxicity Risk from Domoic Acid Exposure (CFSAN)	To correlate pharmacokinetic profiles of single and multiple doses of domoic acid with associated neurohistological and behavioral effects in non-human primates; To identify genetic factors modulating domoic acid sensitivity in Wistar rats; To identify neurochemical biomarkers of domoic acid exposure and damage.
E0693101	**Wilkes** Cairns Chen Fry Heinze Kaysner Lay Miller Rafii Sutherland Turturro Voorhees	Active/ **Chem Lab/** KNLG	First Phase Development of a Rapid Screening Method for Identification of Complex Mixtures by Pyrolysis-Mass Spectrometry with Computerized Pattern Recognition (CFSAN) (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 a 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.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0693201	**Rafii** Cerniglia	Active/ **Micro Lab/** METH	Polymerase Chain Reaction Fragment Length Polymorphism (PCR-RFLP) for Analysis of the Azoreductase Gene of Anaerobic Bacteria Isolated from the Human Intestinal Tract	To study the effects of genetic variations on the metabolic activities of azoreductases from bacteria isolated from human intestinal tract.
E0693301 E0693311 E0693321 E0693331	**Dass** 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 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 or 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** Doerge Freeman Hansen Luo Thompson	Active/ **Chem Lab/** 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 of Veterinary Medicine (CVM). Analytical residues in both the catfish and salmon tissue substrates will be developed.
E0693801	**Evans** Hanna	Active/ **Chem Lab/** METH	Quantitative Determination of Enantiomers Composition and Purity of Drugs by NMR Spectroscopy (ORA)	1) To develop NMR methods to monitor enantiomeric purity of a group of B-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) To 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	Active/ **Bio Tox** AGNT	Preparation of Antibodies Against the C1-C10 and Tricar-ballylic C14-C20 Segments of Fumonisin B_1: Development for Quantitative and Molecular	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 in biologic samples including food.
E0694201	**Zhang** Ali Cerniglia Evans Freeman	Active/ **Micro Lab/** PRED	Microbial Transformations of Antidepressants	To 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	**Frederick** Ali Binienda Gillam Paule Slikker	Active/ **Neuro Tox/** AGNT	Behavioral and Neurochemical Effects of Short Course, High Dose Exposure to Methylenedioxymethamphetamine (MDMA) or dexfenfluramine (FEN) in Rhesus Monkeys	1) To 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). 2) 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. 3) 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. 4) To determine if short course, high dose exposure to MDMA or d-FEN produces long-lasting changes in CNS function by establishing a second acute dose-response curve for each drug after each exposure. 5) 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/ **Chem Lab/** METH	Development of Methods for Analysis and Confirmation of B-Agonists	1) Develop determinative and confirmatory procedures using LC-APCI/MS for multiresidue screening B-agonists in livestock tissues. 2) Develop synthetic procedures to produce authentic B-agonist standards for use in regulatory screening. 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 B-agonists in livestock issues.
E0694601	**Kadlubar** Anderson Potter	Active/ **Mol Epi/** PRED	A Case-Control Study of Pancreatic Cancer and Aromatic Amines	To 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	Active/ **Mol Epi/** PRED	Role of Acetylation and N-Oxidation in Colorectal Cancer	To 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
E0694801	**Dass** Casciano Heflich	Active/ **Gen Lab/** PRED	*In Vitro* Induction of Mutation by Carcinogens in the *HPRT* Gene in Mouse T-Lymphocytes	1) T-lymphocytes will be isolated from the spleen of (unexposed) mice following published procedures. 2) Mutants defective in the *hprt* gene (thioguanine-resistant or TG) will be isolated by a limiting dilution technique. 3) Mutations in the *hprt* gene will be sequenced.

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E0694901 E0694911	**Pipkin** Hinson Lyn-Cook Manjanatha Shaddock	Active/ **Gen Lab/** PRED	The Effect of P53 Null Phenotype on Bleomycin-induced Stress Protein Elicitation *In Vivo* in Transgenic Mice	1) Investigate the structure of the 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 (SP) 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 months of age (during the G1-phase of the cell cycle) by polyacrylamide gell 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. 3) Investigate the phosphorylation patterns of sps as a reflection of gene expression as elicited by BL as in objective 1). 4) To identify and examine nuclear polypetides 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.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0695001	**Manjanatha** Casciano Harris Shaddock	Active/ **Gen Lab/** PRED	Molecular Analysis of *In Vitro* Mutations in the Transgenic Rat2 Cells Exposed to DMBA and Tamoxifen: Comparison of Mutagenesis in the Transgene lacI with the Endogenous Gene *hprt*	1) To 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. 2) To determine the mutant frequency and mutations spectrum of the endogenous *hprt* reporter gene in Rat2 cells following exposure to DMBA and tamoxifen. 3) Compare *in vitro* mutant frequencies and mutational spectra with those determined in the Big Blue rats *in vivo* from Experiment 6906.
E0695201	**Chou** Jackson James Poirier	Active/ **Bio Tox/** CNPT	Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin B1 Induced Carcinogenesis on Male F-344 Rats fed Methyl Deficient Diets	To 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, preneo-plastic foci formation and tumor incidence during the post-initiation stages of AFB1-induced carcinogenesis in male F344 rats. The results of these studies will : 1) test the hypothesis that DR may be an antagonist to the promotional effect of MD in the AFB1-induced carcinogenesis; and 2) evaluate the correlations between the effects of DR and MD on the chemically induced tumor incidence and various bio-markers during the post-initiation stages of carcinogenesis.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0695301	**Young** Bolon Branham Haas Meehan Sheehan Warbritton	Active/ **Biometry/** PRED	Rodent Embryo and Fetal Sectioning for Three Dimensional Image Reconstruction and Animation	To 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.
E0695401	**Freni**	Active/ **Biometry/** CNPT	Resting Metabolic Rate, Body Composition, and Dietary Assessment	1) Develop a validated prediction model for the resting metabolic rate; 2) Collect dietary intake data while maximizing their accuracy; 3) Identify under- or over-reporting of dietary intake data, with emphasis on potential relations between reporting bias and anthropometric data.
E0695501	**Griffin** Gollon Hobbs Kadlubar	Completed/ **Tech Adv*/** AGNT	Accumulation of Manganese in Edible Muscle of Channel Catfish (*Ictalurus punctatus*) Following Exposure to Water	To determine the concentration and retention time of residual manganese in edible muscle of channel catfish after exposure to water borne potassium permanganate.

*Technology Advancement

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E0695601	**Jackson** Weis	Active/ **Repro Lab/** CNPT	An Evaluation of Dietary Fibers for the Prevention of Mammary Cancer in Female Rats	1) To 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) Using this assay, to evaluate several dietary fibers for their ability to increase estrogen excretion and to lower estrogen levels; 3) To test the most effective fibers in the DMBA-mammary tumor model for their ability to inhibit tumor development at dietary levels shown to lower estrogen levels; 4) To establish if the inhibitory effect of dietary fiber on mammary tumor inhibition is dependent on the level of dietary fat.
E0695701 E0695711	**Young** Fleisher Laborde Bolan Hagstrom	Active/ **Biometry/** AGNT	Changes in the Disposition of Methadone in Pregnant Rats and their Fetuses	To 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 Fischer 344 Rats	1) To 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 B1, N-hydro-xy-2-acetylaminofluorene, benzo-(a)pyrene, 2-amino-3-di-methyl-imidazoquinoline, 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; and 3) Determine the types of mutations produced in the *hprt* and *lacI* genes in the mutants induced in Objective 2.
E0695901	**Rafii** Cerniglia Hehman	Active/ **Micro Lab/** METH	Cloning and Characterization of the Genes Involved in the Metabolism of Nitro Compounds by Mycobacterium sp. Pyr-1	To 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.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0696001	**Hansen** Dial Grafton Terry	Active/ **Repro Lab/** PRED	Further Studies on the Mechanism of Valproic Acid Acid-Induced Embryotoxicity	1) Determine a sensitiive 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-adenosyl-methionine/S-adenosylhomocy-steine (SAM/SAHC) ratios in control and VPA-treated embryos during the sensitive period; 4) Determine if VPA produces hypo-methylation of DNA in embryos treated with the drug *in vivo*; 5) Determine if inactivation of methionine synthase increases the embryotoxicity of VPA.
E0696101 E0696111	**James** Miller Pogribny	Active/ **Bio Tox/** AGNT	Mechanisms of Immunotoxicity and Carcinogenicity Associated with Silicone Breast Implants (OWH)	Examine the acute and chronic cellular and subcellular responses to subcutaneous silicone implants utilizing state-of-the-art immuno-histochemistry and molecular biology technologies.
E0696201	**Hammons** Blann Kadlubar Lyn-Cook	Active/ **Mol Epi/** PRED	Methylation Profile, Gene Expression, and Enzyme Activity of *CYP1A2* in Human Livers	To determine the possible involvement of epigenetic mechanisms in the regulation of the CYP1A2 gene. DNA methylation profiles of this gene will be examined and compared with gene expression and enzyme activity in human livers that have been classified by smoking status, gender, and age.

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E0696301 E0696311	**Delclos** Chen 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 Pogribny	Active/ **Bio Tox/** CNPT	Prevention of Ubiquitous Synthesis of the Agouti Protein by Methyl Supplemented Diet	To test the hypotheses that dietary methyl supplements fed to pregnant mice: 1) Affect selected aspects of DNA methylation and phenotypic characteristics dependent on DNA methylation patterns in the offspring; 2) increase the proportion of methylated cytosines in IAP promoter sequences in Avy/a offspring; and 3) increase expression of the agouti phenotype and have no gross morphological effects on the offspring.

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E0696501	**Erickson** Campbell Holland	Active/ **Micro Lab/** METH	Development of an Improved Method for Determining the Tuberculocidal Activity of Chemical Disinfectants for Medical Devices	To 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 quantitation 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 of 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 Interactive Oxidative Effects of Magnesium and Calcium with Selected Heavy Metals	To 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; To investigate the occurrence of adaptive responses in respect to the occurrence of oxidant stress from heavy metal toxicity; To evaluate interactions of the anti-oxidant ascorbate in respect to oxidative damage from selected heavy metals; To gain insight into mechanisms of action in regard to the toxicity and tumorigenic process instigated by heavy metals.
E0696901	**Baker** Medlock Sheehan	Active/ **Repro Lab/** KNLG	Enxymatic Oxidation of 17β-Estradiol: Role of the Products in Hormone Action	To determine how estradiol metabolites formed by peroxidase or tyrosinase interact with the estrogen receptor.

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E0697001 E0697011 E0697021	**Coles** Beland Fullerton Heflich	Active/ **Bio Tox/** CNPT	Sequence Specificity of DNA Adduct Formation and Removal Following Chronic Carcinogen Administration	To 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	Active/ **Biometry/** CNPT	Tree-Structured Over-Dispersed Binomial and Over-Dispersed Poisson Regression Models	1. To develop a tree-structured regression model to analyze over dispersed binomial and over dispersed Poisson data; 2. To develop an algorithm that extends tree-structured regression to the generalized linear regression model; 3. To identify the local effect of the covariates by stratified analysis of the data using tree-structured models; 4. To apply this method to developmental toxicity studies.
E0697201	**Wilkes** Abramson Billedeau Freeman Heinze Pothuluri	Active/ **Chem Lab/** METH	Universal Interface Development and Applications	The ultimate objective of this work is to develop 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.

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 (OWH)	1) To define the ontogeny of insulin-like growth factor (IGF) system mRNA expression in the developing rat uterus; 2) To determine the uterine cell types in which IGF system mRNAs are expressed; 3) To determine the effects of diethylstilbestrol (DES), tamoxifen (TAM), and ICI 182,780 on IGF system mRNA expression at selected developmental stages.
E0697401	**Arani** Chen Freni	Active/ **Biometry/** CNPT	Collinearity Under Proportional Hazards Model	1) To provide diagnostic tools to detect the presence of collinearity under proportional hazards and its quantitative effect on the results; 2) To provide algorithms to combat the harmful influence of collinearity, i.e., stabilize the parameter estimates and their variance. 3) To conduct a simulation study to examine the effectiveness of the algorithms.

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E0697501 E0697511	**Aidoo** Bishop Heflich Lyn-Cook Mittelstaedt	Active/ **Gen Lab/** PRED	The Frequency and Types of Spontaneous Mutations Found in the *hprt* and *lacI* Genes of Lymphocytes from Transgenic Big Blue Rats	1) To determine the frequency of spontaneous mutation at the *hprt* and lacI loci in pre-weanling, young (four-month-old) and old (18-month-old) Big Blue rats; 2) To determine the types of mutations present in the mutants from Objective 1; 3) To compare the results of the analysis conducted from Objective 1 and 2 to determine how well mutational results from the transgenic *lacI* locus predict mutations at the endogenous locus; 4) To compare the results of the *hprt* analysis conducted for Objective 1 and 2 with the results of similar analyses of spontaneous *hprt* lymphocyte mutations conducted in humans to determine how well mutational analysis in the model rat assay predicts mutagenesis in humans.
E0697601 E0697611	**LaBorde** Hansen Hinson Lyn-Cook Pipkin Shaddock	Active/ **Repro Lab/** CNPT	Dose-Response of Retinoic Acid-Induced Stress Protein Synthesis (SPS) and its Correlation with Developmental Toxicity in CD-1 Mice	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 GD11. Determine the incidence of cleft palate on gestation day 17 (GD 17) and the extent of synthesis of Sps in craniofacial tissue determined 2.5 hr after RA treatment following various doses of RA administered on GD 13.

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E0697701 E0697711	**Chen** Burkhart Casciano Heflich Malling	Active/ **Gen Lab/** PRED	Evaluation of Chemical-Induced Mutagenesis in Transgenic Mice Containing the ΦX174 am3	Establish the experimental parameters necessary to demonstrate a mutant frequency of 1.5 to 2 fold above background; Establish the sensitivity of the am3 mouse model to 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; Establish 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.
E0697801	**Ambrosone** Kadlubar Tang Carino	Active/ **Mol Epi/** PRED	Chemical Carcinogenesis: Epithelial Cells in Breast Milk	1) To develop and refine a methodology for separation of luminal epithelial cells from human breast milk for DNA extraction; 2) To detect and quantify aromatic/hy-drophobic-DNA adducts in luminal epithelial cells derived from human breast milk; 3) To detect genetic polymorphisms in carcin-ogen-metabolizing genes derived from DNA extracted from epithelial cells in human breast milk; 4) To evaluate the relationships between carcinogen-DNA adducts and smoking status, and adduct levels with polymorphisms in NAT1, NAT2, GSTM1.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0697901	**Frederick** Gillam Paule	Active/ **Neuro Tox/** PRED	Validation of the NCTR Nonhuman Primate OTB as a Predictor of Neurobehavioral Toxicity II. Pharmacological Manipulation at Specific Neurotransmitter Receptor Subtypes	1) To further explore the extent to which the use of operant behavioral techniques in nonhuman primates can serve to reliably model the effects of compounds selected to act on specific neurotransmitter systems; 2) To 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) To 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) To more thorougly 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) To determine if the acute behavioral effects of the exogenous com-pounds 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** Ang Churchwell Doerge Luo Thompson Wilkes	Active/ **Chem Lab/** METH	Development of Methods for Analysis and Confirmation of Erythromycin A Residues in Tissue Samples from Terrestrial and Aquatic Farmed Animals by Liquid Chromatography	The principal objective of this project is to 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 of Veterinary Medicine.
E0698101	**Cooney** Poirier Wise	Active/ **Mol Epi/** CNPT	Investigation of Short Term Dietary Methyl Supplementation in Manipulation of DNA Methylation and Methyl Metabolism in Mice	To 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. The effects will be determined at two time points and three levels of methyl supplementation. The studies proposed herein will provide data on some molecular and cellular events resulting from methyl supplemented diets. These studies will 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
E0698201	**Allen** Siitonen Thompson	Completed/ **Chem Lab/** METH	Development of Multi-Residue Method(s) for the Determination of Lead and Other Metals in Sweeteners and Edible Oils by Plasma Atomic Emission Spectrometry	Develop method(s) on inductively coupled plasma atomic emission spectroscopy (ICP_AES) for the determination of lead in sweeteners and edible oils at proposed regulatory levels. Methods will be amenable to direct implementation in other laboratories and provide a reduction in time and cost as compared to existing methods.
E0698301	**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 Neurohisto-logical Evaluations in Mouse and Rat Brain (CDER)	1. To determine the effects of ibogaine on dopamine, serotonin and their metabolite concentrations in different regions of mouse and rat brain. 2. To determine the effects of ibogaine on reactive oxygen species (ROS) and lipid peroxidation in different regions of mouse and rat brain. 3. To determine the effects of ibogaine on the activities of several antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and gluthathione levels in different regions of mouse and rat brain. 4. To evaluate the effects of ibogaine on the activity of nitric oxide synthase (NOS) in different regions of the mouse and rat brain. 5. To determine the levels of ibogaine, noribogaine and neurohormone, prolactin and corticosterone in plasma of mouse and rat. 6. To evaluate the neurohistorical effects of ibogaine in different brain regions in the mouse and rat and to correlate them with any neurochemical alterations.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0698401	**Chen** Kodell Zheng	Active/ **Biometry/** METH	Statistical Analysis and Characterization of the Joint Actions of Toxicants	To develop a procedure for analyzing the quantal response data from a mixture experiment at a fixed total concentration; to develop a procedure for analyzing the survival data from a mixture experiment at a fixed total concentration; To develop a mixture model including both proportions and total concentrations; to apply the proportion-concentration model to characterize the joint actions of toxicants.
E0698501 E0698511	**Hansen** Dial Grafton	Active/ **Repro Lab/** PRED	The Role of Reactive Intermediates in Carbamazepine-Induced Embryotoxicity	To determine if the anti-oxidant, glutathione (GSH), is able to decrease CBZ-induced embryotox-icity in mouse embryos; To determine if inhibition of GSH synthesis by L-buthionine-(S,R)-sulfoximine (BSO) increases the embryotox-icity of CBZ; to determine if the antioxidative enzyme, superoxide dismutase (SOD) decreases CBZ induced embryotox-icity; to determine if the prostaglandin H synthase inhibitor, aspirin, decreased CBZ induced embryotoxicity; to determine if treatment with 12-o-tetradecan-oylphorbol-13-acetate (TPA) which activates the release of arachidonic acid decreases CBZ induced embryotoxicity; to determine if treatment with eico-satetraynoic acid (ETYA), an inhibitor of both prostaglandin H synthase and lipoxygenase decreases CBZ induced embryo-toxicity.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0698601	**Delclos** Blaydes Sepai	Active/ **Bio Tox/** CNPT	Serum Albumin Adducts as Markers of Exposure to Toluene-diamines from Implanted Polyurethane Foam	The goal of this experiment is to determine whether this protein-associated toluenediamine accurately reflects exposure to free toluenediamines released from the polyurethane by leaching or by breakdown of the polymer rather than exposure to toluenediamine containing oligomeric breakdown products. In addition to allowing an evaluation of this methodology for the purpose of monitoring exposure to polymer degradation products, these data will be used to help interpret an ongoing study of TDA-plasma protein adducts in German women with polyurethane covered breast implants.
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	Active/ **Micro Lab/** METH	Development of a Universal Protocol for Detection and Identification of 13 Species of Foodborne Pathogens in Foods by Polymerase Chain Reaction (PCR)	Develop 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; and 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 Lab/** METH	Development of Methods for Analysis of Volatile and Nonvolatile N-Nitrosamines in Relevant Cosmetics and Nitrite Cured Meat Products	Develop methods for extraction, cleanup, and anlaysis of non-volatile N-nitrosamines in cosmetics and meat products using combined LC detection methods with confirmation by compatible MS ionization methods; investigate the applicability of LC-ESI/MS and /or LC-APCI/MS as a multiresidue, trace level, quantitative technique for anlaysis 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	To elucidate the role of human cytochrome P450 1B1(*CYP1B1*) in drug metabolism and carcinogenesis. Specific aims are: to design and develop peptide-specific antibodies against human *CYP1B1*; determine the levels of *CYP1B1* protein in various human tissues; evaluate *CYP1B1* expression as a biomarker for tumorigenesis; identify *CYP1B1* inducers among the most common drugs and carcinogens; identify *CYP1B1* substrates, including the endogenous steroid hormones, as well as drugs and carcinogens known to be metabolized by the closely related cytochromes P450 1A1 and 1A2; find specific enzyme inhibitors for *CYP1B1*; develop a sensitive, convenient, and specific assay method for *CYP1B1* enzyme activity *in vitro*; and 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	To 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 bleo-mycin; to 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 to evaluate the integrity of mitochondrial DNA in Bleomycin treated rodents.
E0699201	**Patterson** Binienda Duhart Kim Lipe Slikker	Active/ **Neuro Tox/** PRED	Validation Study of the Physiologically-Based Pharma-cokinetic (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)	To 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 Sams	Active/ **Bio Tox/** KNLG	Evaluation of Host Factors Contributing to Differences in the Response to Biomaterials	The goal of this protocol is to 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
E0699401	**Gazzara** Dow-Edwards Gough Holson	Active/ **Neuro Tox/** CNPT	Efficacies and Relative Potencies of the Monoamine Uptake Inhibitors Cocaine, Fluoxetine and GBR 12909 in Preweanling Male and Female Rats as Measured by Cerebral Microdialysis	To establish the efficacies of three different compounds as reuptake inhibitors of extraneuronal dopamine and/or serotonin *in vivo* in the nucleus accumbens of preweanling male and female rats; to compare the relative potencies with which these compounds inhibit the reuptake of extraneuronal dopamine and/or serotonin *in vivo*; to determine whether or not age and/or sex are factors that alter the efficacies and relative potencies of these compounds as reuptake inhibitors of extraneuronal dopamine and/or serotonin *in vivo*.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0699501	**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 the 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, anti-estrogens, and proges-terones 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 ot 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.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0699601	**Morris** Chen Domon McGarrity	Active/ **Gen Lab/** CNPT	Evaluation of the Genotoxic Potential of Genistein in Human Lymphoblastoid Cells	To confirm the potential mutagenicity of genistein utilizing the *tk/hprt* mutation assay; to determine if apoptosis can account for the toxicity of genistein; to characterize the effect of genistein exposure on the traverse of the cell-cycle; to 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.
E0699701	**Miller** Freeman Heinze Holcomb Lansden Lay Thompson Wilkes	Active/ **Chem Lab/** 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 (decomposition); develop novel rapid detection methods for the determination of analytes in seafood; extension of the microwave mediated distillation-solid-phase extraction (MD-SPE) technologies.
E0699801 E0699811	**Hart** Aidoo Chou Duffy Feuers Fu Hass James Leakey Lu Lyn-Cook Pipkin Turturro	Active/ **Gen Tox/** PRED	Memphis Study: Evaluation of Calorically Restricted Human Surgical Samples Received from Dept. Of Surgery, University of Tennessee, Memphis	Determine whether rodents and humans behave biologically in the same manner when calorically deprived but nutritionally supplemented.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0699901	**Kim** Cerniglia	Active/ **Micro Lab/** PRED	Biochemical and Molecular Analysis of Polycyclic Aromatic Hydrocarbon (PAH) Degradation by Bacteria	1. To characterize multiple genes for the initial aromatic dioxygen-ase from F. Yanoikuyae B1; 2. to determine putative common roles of ferrodoxin and reductase components of initial dioxygenase in mono- and polycyclic aromatic hydrocarbon degradation; 3. to determine roles of the NahD (2-hydroxychromene-2-carboxylate isomerase) and NahE (cis-o-hydroxybenzylidenepyruvate aldolase) in polycyclic aromatic hydrocarbon degrada-tion by S. Yanoikuyae B1; 4. to determine molecular basis for polycyclic aromatic hydrocarbon degradation by *Mycobacterium sp.* PYR-1.
E0700101	**Nawaz** Cerniglia Khan	Active/ **Micro Lab/** 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.
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	To 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 the deletion of an extended sequence. Phage harboring these mutations will be used to construct a transgenic mouse model for measuring mutations *in vivo*.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0700301	**James** Hart Muskhelishvili Pogribny	Active/ **Bio Tox/** CNPT	Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy	1. In N-methyl-nitrosourea (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. to 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 Peroxidation and Endogenous DNA Adduct Formation from Chloral (CFSAN)	1. To develop analytical methodologies for analysis of lipid peroxidation products and endogenous DNA adducts; 2. To determine whether or not the drugs of FDA interest, including benzodia-zepines and antihistamines studied in E687901, and other chemicals induce lipid peroxidation and endogenous DNA adduct formation in vitro; 3. To determine the inhibitory effect of lipid- and water-soluble antioxidants on drug-induced lipid peroxidation and endogenous DNA adduct formation in vitro; 4. To determine whether or not the malondial-dehyde-modified MG-1 DNA adduct and/or other endogenous DNA adducts can be used as biomarkers of lipid peroxidation; 5. *Salmonella typh-imurium* TA 104 and determine whether or not mutagenicity in *Salmonella typhimurium* TA 104 and determine whether or not mutagenicity in *Salmonella typh-imurium* TA 104 can be used as a biomarker of lipid peroxida-tion induced by chemicals that generate free radicals upon metabolism.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0700501	**Lay** Darsey Heinze Holland Miller Rafii Sutherland Voorhees Wilkes	Active/ **Chem Lab** METH	Rapid Identification of Intact Whole Bacteria Based on Spectral Patterns Using MALDI-TOF MS (CFSAN)	1. To evaluate the 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. To establish a standard set of conditions for the acquisition of MALDI/TOF mass spectra from bacteria suitable for use in bacterial identification. 3. To 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. To 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. To evaluate the use of mass spectral recognition techniques of the identification of bacteria from mixtures based on MALDI-TOF MS analysis of the mixtures; 6. To determine the minimum number of bacteria necessary for obtaining standard mass spectra; 7. To evaluate the effects on the reproducibility of spectra obtained from whole bacteria under different conditions of sample handling, storage, and cell growth.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0700601	**Gehring** Churchwell Cooper Doerge Holcomb Rushing Thompson	Active/ **Chem Lab** METH	Development of Multiresidue Methods to Determine and Confirm Sulfonamides in Edible Tissues of Aquacultured Species	The objective of this project is to 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 postcolumn derivatization and fluorescence detection for the determinative procedure and liquid chromatography with atomospheric pressure chemical ionization mass spectrometry (LC-APCI/MS) for the confirmatory procedure.
E0700701	**Rafii** Cerniglia Sutherland	In Review/ **Micro Lab**/ CNPT	Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds (OWH)	1. Detection of various metabolites of phytoestrogens, produced by the metabolism of these compounds by human intestinal bacteria of adults and infants, 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 merabolites from phytoestrogens and elucidation of enzymes involved in various steps of these metabolic processes; 4. The effects of phytoestrogens and their metabolites on the population, composition, metabolic activity and enzyme production of bacteria from the human gastrointestinal tract.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0700801	**Sheehan** Branham Hass	Active/ **Repro Lab** AGNT	Bioassay of Reproductive Tract Toxicities Caused by Genistein and Methoxychlor in Sprague-Dawley Rats (OWH)	To 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 continous feeding protocol will be compared to a 5-day injection of protocol at 4 periods of development.
E7000901	**Arani** Chen	Active/ **Biometry** METH	Analysis of Multiple Tumor Sites	1. To develop analytical and numerical techniques for computing the experiment-wise error rate in testing of multiple tumor sites; 2. To 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. To evaluate the experiment-wise error rate and power of global statistics for an overall test of carcinogenicity; 4. To recommend optimal procedures, which control the experiment-wise error rate and still maintain the power, for the analysis of multiple tumor sites.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
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 (CFSAN)	1. To evaluate the acute effects of the mitochondrial inhibitor 3-NPA on brain metabolic activity using electrophysiological, neurochemical, and neurohistological endpoints: a) spontaneous electrical bain 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 detectible neuronal damage; 2) To assess the possible neuroprotective effect of L-carnitine in the rat model of 3-NPA induced histotoxic hypxia.
E0701101	**Beland** Marques	Active/ **Bio Tox** AGNT	DNA Adducts of Tamoxifen (OWH)	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.
E0701201	**Wolff** Tolleson	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	To determine whether or not the agouti protein stimulates mitogenesis in vitro.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
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 Mechanims of Action of Neuro-toxicants	To 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 neurotoxiants; 2. To 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.
E0701401	**Aidoo** Desai Lyn-Cook Manjanatha 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 Radiomimetic Antitumor Drug Bleomycin	1. To determine the gentoxic activity of dimethylbenz(a)anthra-cene (DMBA) and bleomycin (BM) by the cytokinesis-block micronucleus and *hprt* assays in Fischer 344 rat that have been given a mixture of vitamin C, vitamin E and B-carotene and selenium by gavage; 2. To determine the mechanism underlying the inhibitory action of the dietary antioxidant 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 glutathioneS-transferase during DMBA and BM exposures.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0701501	**Ambrosone** Stone Carino	Active/ **Mol Epi** METH	Breast Cancer in African-American Women: Metabolic Modification of Dietary and Hormonal Risk Factors (OWH)	In this study, we intend to examine the role of interindividual 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	Active/ **Bio Tox** CNPT	Molecular and Metabolic Determinants of Maternal Risk and Progression of Down Syndrome: Potential for Nutritional Interventions (OWH)	1. To 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. To 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
E0701701	**Lyn-Cook** Blann Hammons Kadlubar	Active/ **Mol Epi** PRED	The Effects of Low Zinc Levels on *ras*, mdr-1 Genes Activation and on Metabolic Enzume Activities in Normal and Neoplastic Human Pancreatic Cells: A Possible Risk Factor for Pacreatic Cancer	The major objective of this proposal is to determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*. The final objective of this study is to 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** Dass Heflich	Active/ **Gen Lab** PRED	Validation of the Mouse Targeted tk+/- In Vivo System for Use in Mutagenicity Studies	1. To expand a colony of transgenic *tk+/-* mice using breeding of *tk+/-* founders and C57B1/6 mice, and to transfer the *tk+/-* genotype to a C57B1/6 background; 2. to determine spontaneous mutant frequencies at the *tk* and *hprt* loci of splenic T-lymphocytes for mice of different ages; 3. to induce mutations in *tk+/-* transgenic mice using treatment with the point mutagen N-ethyl-N-nitrosourea (ENU) and the clastogens Bleo-mycin (BLM) and γ-radiation, and to measure the kinetics of mutant induction at the *tk* and *hprt* loci; 4. To breed transgenic *tk+/-* parents in an attempt to derive *tk=/-* knockout mice, and study the biological significance of the tk gene in mice; 5. to determine how the *tk=/-* genotupe may effect mutant frequencies at the *hprt* locus.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
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	To induce an experimental autoimmune prostatitis in male rhesus monkeys by immunizing animals with homgenates of monkey prostate gland admixed with Freund's adjuvant; 2. to identify the target proteins of the induced autoimmune prostatitis by using the immune sera (IgG) from the above animals to i) screen prostate homogenates by Western im-munoblot analyses and ii) screen a monkey prostate cDNA expression library.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0702001	**Hansen** Grafton Streck	Active/ **Repro Lab** PRED	Antisense Knockouts of Genes in the Folate Pathway and Effects on Neural Tube Development	1. To determine if knocking out 5,10-methyltetrahydrofolate reductase (MTHFR) activity in mouse embryos in vitro produces neural tube defects; 2. to 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. to 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. to determine if knocking out methionine synthase (MS) activity in mouse embryos in vitro produces neural tube defects; 5. to 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. to determine if exogenous vitamin B_{12} is able to overcome the lack of MS activity and produce closed neural tubes in mouse embryos treated in vitro; 7. to determine if knocking out methionine adeno-syltransferase (MAT) activity in mouse embryos in vitro produces neural tube defects; 8. to 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. to determine if addition of exogenenous 5-methyltetrahydro-folate is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated in vitro.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0702101	**Ambrosone** Green Hine Kadlubar Lang Stone Carino	In Review/ **Mol Epi** METH	Deteminants of Indolent and Invasive Prostate Cancer	1. Determine levels of carcinogen exposure in African Americans and Caucasians with histologially 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 expolsure data obtained in Objective 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 Objectives 1 and 2.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0702201	**Muskhelishvili** Streck Tolleson	In Review/ **Pathology** CNPT	Determination of the Role of Insulin-like Growth Factor I Receptor Signaling System in Dietary Restriction-induced Upreg-ulation of Apoptosis in Rat Liver Microenvironment	1. To determine whether or not DR reduces the ability of transplanted BAG2-GN6TF cells (a rat liver epithelial tumor cell line) to form tumors in a liver microen-vironment and if this is due to upregulation of apoptosis. 2. To determine whether or not expression of IGF-I and IGF-IR mRNAs is reduced in the livers of DR rats relative to their ad libitum-fed (AL) counterparts. 3. To determine whether or not expression of IGF-IR mRNA and protein in BAG2-GN6TF cells is reduced after their transplantation into the livers of DR rats relative to those in AL rats. 4. To determine whether or not a decrease in the number of IGF-IRs in BAG2-GN6TF cells before transplantation will diminish the ability of BAG2-GN6TF cells to form tumors in the livers of AL and DR rats.
E0702301	**Tolleson** Howard Jenkins Leakey Morris Rowland	In Review/ **Bio Tox** METH	The Role of Human Metabolism in Endocrine Disruption (OWH)	Humans may be exposed to compounds in the diet or in the environment that disrupt endogenous endocrine responses in various tissues. We propose to utilize cell biological approaches to determine the role of human cytochromes P-450, UDP-glu-curonosyltransferases, and sulfo-transferases in the antiestro-gens. 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.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0960001	**Cooney** Poirier Wise	Completed/ **Mol Epi/** AGNT	Preliminary Assessment of Methyl Supplements, Metabolism and DNA Methylation	Make a preliminary assessment of whether or not dietary methyl supplements in adult mice and rats will effect short term changes in levels of methylation metabolites or levels of DNA methylation. Dietary methyl supplements can signficantly improve methyl availability in humans even when the origin of reduced methyl availability is genetic. This project will also provide assessment of methodologies for collecting multiple animal organs for analysis of labile molecular components.
P00268	**Flammang** Casciano Chen Freni Kodell	Active/ **Off Res*/** RSUP**	NCTR/FDA Integrated Research (CFSAN) (CDER) (CDRH) (CVM) (CBER)	Interaction with and development of collaborative projects with other FDA Centers.
P00338	**Turturro**	Active/ **Biometry/** METH	Training for Special Employment Program (Foreign National) in the PCR	To provide training for individuals in the Foreign National Training Program in the PCR.

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**Research Support

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
P00358	**Sheehan** Branham Burroughs Medlock	Active/ **Repro Lab/** KNLG	Training in the Estrogen Developmental Toxicity Bioasssay (OWH)	This training protocol is the first phase of a project funded by the Office of Women's Health, FDA. In order to carry out the study, Pathology Associates, Inc. (PAI) need to be trained in animal sacrifice, tissue removal and processing, instrumentation, morphome-tric techniques, aspects of project management and procedures of data collection, recording, retrieval, reduction, and summarization.
P00364	**Beland** Marques	Completed/ **Bio Tox/** AGNT	DNA Adducts of Tamoxifen (OWH)	Preparation of a-hydroxytamoxi-fen and 4-hydroxytamoxifen synthetically.
P00365	**Sams** Delclos	Completed/ **Bio Tox/** METH	Oxidative Stress-Related DNA Damage and *hprt* Mutations in the Rodent Air Pouch	1) Developing a means of ensuring uniform exposure of the pouch lining to materials introduced directly into the pouch; 2) determining the time of maximal DNA damage; and 3) establishing conditions, based on the literature, for the culture of fibroblasts from the air pouch lining.
P00369	**Sheehan** Crews	Completed/ **Repro Lab/** KNLG	A Biologically Based Dose Response Model for Estradiol Effects on Sex Determination in Turtles	To experimentally test the hypothesis that if an endogenous hormone is causing an effect in a population, then treament with any dose of the same chemical cannot show a theshold because the threshold dose is already exceeded in the untreated animals.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
P00370	**Norris** Baker Gaylor Harrison Lay Perkins Sheehan Shvets Strelitz Ulmer	Active/ **Dep Dir/** KNLG	Development of an Estrogen Knowledge Base for Research and Regulation	The purpose of this effort is to 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 quantitative structure activity relationship (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. The result of this effort will be an estrogen database with predictive capability.
P00376	**Hansen** Streck	Completed/ **Repro Lab/** PRED	Development of Limb Bud Culture System	To develop a limb bud organ culture system for future molecular biology experiments - this project is a prelude to proposed project X70003.
P00377	**Aidoo** Montgomery	Completed/ **Gen Lab/** AGNT	Training for Mutagenesis Studies for ORISE Internship Program Participant	Training program to enable ORISE program participant to conduct mutagenesis studies with four food derived mutagens.
P00379	**James**	Completed/ **Bio Tox** CNPT	Mechanisms of Immunotoxicity and Carcinogenicity Associated with Silicone Breast Implants	Animals requested for this project are to be used for training of the techniques to be used in E06961.11 which could save much time and expense.
P00380	**Tolleson** Howard Jenkins Leakey Morris Rowland	Active/ **Bio Tox** METH	Development of In Vitro Human Cell Culture Systems to Screen Compounds Suspected to have Estrogenic Antiestrogenic Activity (OWH)	To obtain preliminary results to support the OWH proposal. This study is designed to generate the molecular biological systems necessary to begin evaluating the role of human cytochromes p450, UDP-glucuronosyltransferases, and sulfotransferases in modulating the estrogenic nature of putative endocrine disrupting agents.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
P00381	**James**	Completed/ **Bio Tox** CNCT	Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy	To determine appropriate dose for the chemotherapeutic agent, 5-florouracel (5-FU) to be used in our grant-funded AICR project (E0700301).
P00382	**Hinson**	Completed/ **OMFRS***	PAG for Evaluation of Laboratory Maintenance Contract Proposals	To evaluate this PAG contract.
P00383	**Lomax**	Completed/ **Path** RSUP**	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.
P00384	**Paule**	Active/ **Neuro Tox** RSUP	Develop Additional MBS Task (Titrated IMP)	Develop new titrated IMP task. This task will be similar to the IMP task currently being developed except for the time delay which will be increased for each long and short delay. Plans ar being examined to implement this new task into the IMP which is currently being designed.
P00385	**Hass** Branham Sheehan	Active/ **Repro Lab** KNLG	Validation of the Rat Estrogen Receptor Competitive Binding Assay	To validate the RERCB assay in our laboratory as a basis for providing later data for the Estrogen Knowledge Base.

*Office of Management, Facilities & Research Support

**Center Research Support

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 involves an empirical investigation of OTB performance by normal children and children identified as expessing specific clinical diagnoses including Attention Deficit Disorder with or without Hyperactivity. Project will also cover R.O.W. Task Order #22 - set up to provide statistical support to analyze data collected from tests utilizing the operant behavioral panel at the Ark. Children's Hospital.
P00388	**Streck**	Active/ **Repro Lab** PRED	Identification of Molecular Markers of Peroxisome Proliferator-Activated Receptor-Gamma Activation in the Rat Fetus	1. To determine which rat fetal tissues express peroxisome proliferator-activated receptor (PPARY). 2. To identify, from a set of genes regulated by PPARY in adults, genes that are coex-pressed in the same fetal tissues as express PPARY.
P00389	**Shaddock**	Active/ **Gen Lab**/ PRED	Demonstate and Train Visiting Scientists in *In Situ* Perfusion, Isolation, and Primary Culture of Rat Hepatocytes	To demonstrate and train visiting scientists in the procedures utilized in our laboratory associated with the *in situ* perfusion, isolation, and primary culture of rat hepatocytes.
P00390	**Howard** Melchior	Active/ **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 simlated via a PBPK model. Initially the literature data will be limited to dexamethasone, cocaine, and methyl-mercury. Species comparisons will be made utilizing this single pharmacokinetic model.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
S00006 E0002200 E0010900 E0011000 E0011100 E0011200 E0011300 E0014500	**Campbell**	Active/ **Micro Lab/** RSUP	General Microbiological Support - Bacteriology, Parasitology, Mycology, and Virology	To provide general microbiological support services.
S00032	**Kodell** Freni	Active/ **Biometry/** CNPT	Interagency Projects	To participate on interagency committees and workshops, e.g., EPA, DHHS, NIEHS, and NSTC, concerning risk assessment issues.
S00059	**Allaben**	Active/ **Bio Tox**/ RSUP	INTOX Program	
S00064	**Campbell**	Active/ **Micro Lab/** RSUP	Microbiology Division-media Preparation	To provide microbiological media.
S00116	**Kodell** Chen Freni	Active/ **Biometry/** CNPT	Risk Assessment (General) (CFSAN) (CDRH) (CVM)	Efforts in the improvement of Risk Assessment.
S00137	**Hansen**	Active/ **Repro Lab/** RSUP	CD-1 Mouse Breeding Colony	This colony permits the continued maintenance of these mice resulting in large monetary savings over ordering the mice from outside sources. It also facilitates tracking of animal usage and ensures adherence to AAALAC/ACUC guidelines.
S00138	**Paule**	Active/ **Neuro Tox/** METH	Nonhuman Primate Operant Behavior Training and Maintenance	To 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.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
S00143	**Duffy** Ali Feuers	Active/ **Repro Lab/** METH	Development of an Automated Repetitive Blood Sampling System to Measure Circadian Rhythms Of Blood Chemistries	The mechanism of CR action appears to involve effects on the endocrine system. Measurement of these effects is complicated by investigator-generated disruption of animal homeostasis. The system will provide automatic sampling of true levels of sensitive hormonal parameters.
S00170	**Rafii**	Active/ **Micro Lab/** RSUP	Animals for Antibody Production	To provide antibodies for microbiology program research efforts.
S00174	**Kodell** Chen Collins Freni Alderson Jacobson Pohland	Active/ **Biometry/** METH	Modification and Application of Quantitative Risk Assessment Techniques to FDA Regulated Products (CDER) (CDRH) (CFSAN) (CVM)	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 "safe" conditions of exposure to toxic substance.
S00175	**Kodell** Chen	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.
S00185	**Campbell**	Active/ **Micro Lab**/ RSUP	Special Epidemiology Investigations of Potential Microbiological Contamination Problems	To investigate potential microbiological contamination problems. To report non-routine sample time which is not recorded on Sample Collection Report.
S00189	**Holland** Chamberlain	Active/ **Micro Lab/** METH	Tuberculocidal Efficiency of Various Disinfectants (CDRH)	Assess, modify and validate the AOAC tuberculocidal test procedures for use with disinfectants.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
S00195	**Slikker** Paule Slikker	Active/ **Neuro Tox**/ KNLG	Analysis of Data Relationships for Glutamate Receptor: Phase 1	1. To determine how data related to the GR can be organized, displayed, maniplated, and queried in toxicological contexts. To develop a GR information schema that will lay the foundation for a knowledge mining effort in Neu-rotoxicology and that will support both researchers and reviewers.
S00197	**Lay**	In Review/ **Chem Lab**/ METH	Chemistry Support for ORA's Jefferson Regional Laboratory's TCDD Analysis Program	FDA's needs for the analysis of Dioxins in a timely manner requires chemistry support for ORA's Jefferson Regional Laboratory's TCDD Analysis Program.
S00198	**Beland**	In Review/ **Bio Tox**/ AGNT	In Vivo DNA Adduct Standards	Ongoing support in collaboration with IARC
X00006	**Leakey** Hart Seng	Proposed/ **Chem Lab/** CNPT	Hypothalmic-Pituitary-Adrenal Axis/Hepatic Rats	To understand the role pancreatic polypeptide, corticosterone, trans-cortin, CRF, leptin, insulin, eicosanoids and lipocortins play in mediating the effects of caloric restriction in rats.
X30029	**Slikker** Gaylor Sobotka	Proposed/ **Neuro Tox/** METH	Risk Assessment of Neuro-toxicants	To develop and validate quantita-tive, biologically-based risk assessment procedures for neurotoxicants.
X40057	**Turturro**	Proposed/ **Biometry/** CNPT	Effects of Food Restriction on Risk Assessment	Construct risk assessment models which are able to estimate the impact of caloric restriction on spontaneous and induced carcinogenesis.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X50010	**Ali** Duhart Hussain Lipe Meng Newport Schmued Slikker	Proposed/ **Neuro Tox/** AGNT	Iron Induced Oxidative Stress	To determine the role of dietary iron on the occurence of oxidative stress in the rodent central nervous system.
X60021	**Shaddock** Casciano Harris Manjanatha Pipkin	Proposed/ **Gen Lab/** METH	A Study of Several Hepato-carcinogens in the Rat Hepatocyte System: The Effect of AFB1, 2-AAF, Clofibrate and Methapyrilene 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). Components of the NCTR Operant Test Battery will be used to assist in the clinical assessment of ADHD status.
X70004	**Streck** Webb Young	Proposed/ **Repro Lab/** CNPT	Retinoic Acid Receptor Expression	Project under development.
X70005	**Ferguson**	Proposed/ **Neuro Tox/** KNLG	Neonatal Glucocorticoid Exposure	Project under development.
X70010	**Howard**	Proposed/ **Bio Tox**/ METH	Isolation of Human Genomic Ceramide Synthetase	

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X70026	**Manjanatha**	Proposed/ **Gen Lab/** PRED	A Study of DNA Repair in the Transgene of Big Blue Rats	Project under development.
X70033	**Shuttleworth** Cerniglia Hansen	Proposed/ **Micro Lab/** PRED	Effects of Physio-chemical Factors on the Metabolic Potential of the Drug Metabolizing Fungus, *Cunning-hamella elegans*	The objectives of this study are to 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. This study will provide 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 Gaylor Zheng	Proposed/ **Biometry/** KNLG	A New Strategy for Detecting Carcinogenicity	Project under development.
X70049	**Sutherland** Castlebury Cerniglia Freeman Holcomb Williams	Proposed/ **Micro Lab/** AGNT	Methods for Detection of Beauvericin and Moniliformin	Development of HPLC and capillary electrophoresis methods for detection of the Fusarium mycotoxins, beauvericin and monilifor-min in foods.
X70053	**Turturro**	Proposed/ **Biometry/** AGNT	Model Toxic Response Using Neural Networks	Project under development.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X70059	**Lu**	Proposed/ **Repro Lab/** AGNT	Investigation of Reproductive and Developmental Toxicity of Tamoxifen by Flow Cytometric Cell Cycle Analysis	Project under development.
X70060	**Attwood**	Proposed/ **Res Sup**/ CNPT	NIA IAG (PCR)	Project under development.
X70082	**Delclos**	Proposed/ **Bio Tox**/ AGNT	The Effects of Dietary Genistein on the Growth of Chemically-Induced Mammary Tumors in a Postmenopausal Rat Model	To 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.
X80001	**Rafii** Cerniglia	Proposed/ **Micro Lab**/ KNLG	Importance of Human Intestinal Microflora in Conversion of Phytoestrogenes to Estrogenic Compounds	Project under development
X80002	**Wang** Cerniglia	Proposed/ **Micro Lab**/ METH	Development of Vaccine for *Holieobacter Pylori* and *Vibrio cholorea*	Project under development
X80003	**Delclos**	Proposed/ **Bio Tox**/ CNPT	Endocrine Disruptor Effects on the Metabolism of Endogenous Steroids and Xenobiotics	Project under development
X80004	**Roberts**	Proposed/ **Bio Tox**/ CNPT	Immunochemical Evaluation of Oxidative Damage and Autioimmunity	Project under development
X80005	**Delclos**	Proposed/ **Bio Tox**/ AGNT	The Distribution and Metabolism of Genistein in CD	Project under development

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X80006	**Delclos**	Proposed/ **Bio Tox**/ AGNT	Local and Systemic Effects of Estrogenic Compounds that are Potentially Released from Biomaterials	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
X80008	**Bowyer** Davies Gough Newport Peterson Schmued Slikker	Proposed/ **Neuro Tox**/ CNPT	Evaluation of the Neurotoxic Effects of Limbic Seizure Activity Induced by Amphetamine and Related Compounds	1. To measure the effects of dose and age on the susceptibility of amphetamine-induced limbic-type seizures in two different strains of rat and mouse, and identify areas in the brain, in particular the limbic system, where cell death and neuroplastic changes occurs after amphetamine-induced seizures; 2. Determine the seizuregenic capabilities of amphetamine, fenfluramine, 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 prior to and during seizures induced by amphetamines; 4. Elucidate the role the noradrenergic system plays in seizures generated by amphetamine.
X80012	**McClure** Ambrosone Fu Glasier James Kadlubar	Proposed/ **Mol Epi**/ PRED	Quantification of Chloral Hydrate Induced Lipid Peroxidation in Children	Project under development
X80014	**Hansen** LaBorde	Proposed/ **Repro Lab**/ CNPT	In Vitro Embryotoxicity of Genistein	Project under development

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X80015	**Streck** Webb	Proposed/ **Repro Lab**/ PRED	Growth Factor and Antisense Injections	Project under development
X80016	**Streck** Webb	Proposed/ **Repro Lab**/ CNPT	Developmental Toxicology of Antidiabetic Drugs	Project under development
X80017	**Sheehan** Dial Molan	Proposed/ **Repro Lab**/ KNLG	Tamoxifen and Analogue Effect on Utrine Growth Responses and Apoptosis	Project under development
X80018	**Sheehan** Branham Dial Moland	Proposed/ **Repro Lab**/ CNPT	Bisphenol: A Study in Collaboration with the Plastics Industry	Project under development
X80019	**Sheehan** Branham Dial Moland	Proposed/ **Repro Lab**/ KNLG	Study of a Threshold for Estrogen Action in OVX/ADX Neonates	Project under development
X80020	**Delongchamp** Chen Lung-An	Proposed/ **Biometry**/ PRED	A Mixture Model for Classifying *CYP1A2* Variants	Project under development
X80021	**Delongchamp** Kadlubar Lang	Proposed/ **Biometry**/ METH	An Investigation of Mode Tree Methods	Project under development
X80022	**Zheng**	Proposed/ **Biometry**/ METH	Linking PBPK and BBDR Models	Project under development
X80023	**Poirier** Wise	Proposed/ **Mol Epi**/ PRED	Homocystine Toxicity	Project under development
X80024	**Poirier** Wise	Proposed/ **Mol Epi**/ CNPT	DNA Melose Methylation	Project under development
X80025	**Scallet** Ye	Proposed/ **Neuro Tox**/ METH	Development of an Assay for HACCP Regulation of Transmissible	Project under development

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X80026	**Ang** Lay	Proposed/ **Chem Lab**/ AGNT	Determination of Active Ingredients in Dietary Supplements: I. Salicin and Related Compounds in Botanical Products	The principal objective of the proposed research is to develop sensitive analytical multi-component methods for the determination of salicin and related compounds in botanical dietary supplements regulated by FDA. This work will be conducted in collaboration with CFSAN.
X80027	**Wolff** Cooney	Proposed/ **Bio Tox**/ METH	An Efficient Animal Model for Detecting Maternally Inherited Multigenerational Effects of Nutritionals and Xenobiotics	Multigenerational effects are of great importance for women's health. A mouse system will allow rapid screening of offspring to detect multigenerational effects on phenotype and 5MC of maternal exposure to nutritionals and xenobiotics.

INTERAGENCY AGREEMENTS

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Cooperating Organization: Environmental Protection Agency - Office of Toxic Substances

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0663807	**Laborde** Dial Hansen Harmon Holson Jones Leakey Sheehan Webb	Active/ **Repro Lab/** CNPT	Biologically-Based Dose Response Models for Developmental Toxicity: Effect of Dexamethasone (DEX) During Mid- & Late Gestation on CD Rat Dams & on Fetal Amniotic Fluid, Liver Glycogen Concentration and Enzyme Activity	1. To determine the effect of DEX exposure at two gestational periods (GD 9-14 or GD 14-19) on content and volume of amniotic fluid, and biochemistry of liver. 2. To determine the effect of late DEX exposure (GD 14-19) on maternal urine and blood chemistry. 3. To correlate DEX effects on amniotic fluid and on liver function with the stunting, clefting and wavy ribs produced in rat fetuses by this drug. 4. To assess the degree to which DEX-induced anorexia contributes to any of the above effects.
E0663812	**Young** Hansen Holson Pearce Sheehan	Active/ **Biometry**/ CNPT	Biologically-Based Dose Response: The Pharmacokinetics of Dexamethasone in the Albino Rat Dam and Fetus	To determine 1) the pharmacokinetic profile of DEX in pregnant rat dams after single or multiple exposure to DEX, 2) determine the DEX levels in fetal and maternal tissues after multiple exposures to DEX, and 3) assess the correlation between DEX pharmacokinetic parameters and DEX-induced cleft palate incidence and growth stunting in fetuses.

Cooperating Organization: Environmental Protection Agency - Office of Toxic Substances

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0667800	**Fu** Dooley Kadlubar	Active/ **Bio Tox/** PRED	Neonatal Mouse Bioassay of Eight Complex Mixtures and Three Positive Control Samples	1. To use neonatal male B6C3F1 mice to determine the tumorigenic activity of eight complex mixtures samples (i.e., smoky coal, aluminum smelter emissions, ambient air, cigarette smoke, coke oven emissions, diesel exhaust, polyethylene incineration and roofing tar), three positive control samples (i.e., benzo(a)pyrene, 6-nitrochrysene, and 4-aminobiphenyl) and their carrier (DMSO). 2. To remove target tissues from treated animals and send to EPA for carcinogen-DNA adduct quantitation and characterization. 3. To prepare appropriate synthetic standards for carcinogen-DNA adduct detection.
E0667820	**Fu** Dooley Kadlubar	Active/ **Bio Tox/** AGNT	Mouse Skin Tumor Initiating Activity of Two Ambient Air Samples	Use female Sencar mice to determine the skin tumor initiating activity of two organic extracts of ambient air particulate matter, provided by EPA.

Cooperating Organization: National Institute on Aging

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0049400	**Turturro**	Active/ **Biometry/** CNPT	Caloric Restriction in Fischer 344 Rats	Breed and age Fischer 344 calorically restricted rats.
E0050100 thru E0050805	**Turturro** Hart	Active/ **Biometry/** CNPT	NIA-IAG Caloric Restriction and Aging	Breed and age calorically restricted rodents.

Cooperating Organization: National Institute on Aging

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0050900	**Turturro** Gaylor Hart Sheldon	Active/ **Biometry/** CNPT	F-344 Rat Fed Ralston-Purina Masoro Mod. Diet	Selected diet for caloric restriction.
P00304	**Turturro**	Active/ **Biometry/** AGNT	NIA IAG Administrative Overhead	General administration of PCR IAG.

Cooperating Organization: National Institute of Drug Abuse

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0663306	**Paule** Binienda	Active/ **Neuro Tox/** AGNT	ADDEND: Preliminary Studies for Determining the Effects of Chronic Cocaine Exposure during Pregnancy on the Behavior of Offspring in Monkeys	Increase the number of offspring in the total gestational exposure (TGE) group to ten. Requesting that 10 nonpregnant animals be maintained under chronic cocaine treatment while they are in the breeding program until at least 10 viable offspring are available. Requesting another 7 animals for inclusion in control group to bring the total to 10.
X60048	**Paule**	Proposed/ **Neuro Tox/** AGNT	Grant Proposal to NIDA	Proposed grant with NIDA to eventually be funded via IAG mechanisms. Continuation of E06633 protocols.

Cooperating Organization: National Institute of Environmental Health Sciences

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0250001 E0250011	**Schmued** **Sandberg** Appel Blann Bo Lyn-Cook Paule Schmued Slikker	Active/ **Neuro Tox/** METH	Preliminary Assessment of a Method for Screening the Potential Neurotoxic Effects of Anti-HIV Therapeutics Using Auto-radiographic Measurement of Cellular Metabolic Markers (CBER) (CDER)	To examine, validate and compare the utility of a number of biological markers (2'3'-d-dideoxycytidine (ddC), 2,3'-d-dideoxyinosine (ddI), isoniazid) of neurotoxic insults using *in vivo* and *in vitro* autoradiographic methods.
E0250101	**Patterson** Binienda Slikker Sandberg Lipe Gillam	Active/ **Neuro Tox/** AGNT	Placental Transfer and Fetal Distribution of the Human Immunodeficiency Virus (HIV) Therapeutics: 3'-azido-2',3'-dideoxythymidine (AZT), 2'3'-dideoxyinosine (ddI), and 2',3'-didehydro-2'3'-dideoxythymidine (d4T) (CDER)	To determine the placental and fetal distribution of AZT, ddI and d4T, and their phosphorylated metabolites in the later-term rhesus monkey.
E0250201 E0250211	**Patterson** Paule Sandberg Schmued Sheevers Slikker Zielinski	Active/ **Neuro Tox/** PRED	Neurotoxicological and Behavioral Assessment of the Human Immunodeficiency Virus (HIV) Suppressors 2',3'-dide-oxycytidine (ddC) and Thalidomide in Rhesus Monkeys (CDER)	To assess the neurotoxicity and neurobehavioral effects of chronic treatment with the anti-HIV agents 2',3'-dideoxycytidine (ddC) and thalidomide in rhesus monkeys.
E0250301	**Schnellmann**	Active/ **Neuro Tox/** PRED	In Vitro Cellular Toxicity Studies of the Dideoxy-nucleoside Antiretro-virals, ddI, ddC, 3TC and d4t	1. To utilize an established cell model for investigation of ddN-induced peripheral neuropathy; 2. To examine the metabolism of various ddN's in the PC12 cell model; 3. To evaluate the effect of sublethal concentrations of ddN's on cell functions; 4. To determine the relative contribution of identified ddN-induced alterations in cellular functions to toxicity.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0210001	**Fu** Casciano Heinze Kadlubar Von Tungeln Contrera	Completed/ **Bio Tox/** AGNT	Metabolism and DNA Binding of Chloral Hydrate (CDER)	1. Characterize and quantify the metabolites of chloral hydrate 2. Determine the mechanism of metabolic activation of chloral hydrate 3. Prepare synthetically carcinogen modified DNA adduct(s) of chloral hydrate and its metabolites 4. Determine the principal metabolizing responsible for metabolic activation 5. Study mutagenicity, metabolism and DNA adduct formation of chloral hydrate and its metabolites.
E0210101	**Beland** Contrera Dooley	Completed/ **Bio Tox/** AGNT	Fourteen-day, Repeat-dose, Range-finding Study of Chloral Hydrate in Male and Female B6C3F1 Mice (CDER)	To determine the doses of chloral hydrate to be used in a chronic study.
E0210201	**Beland** Contrera Dooley	Completed/ **Bio Tox/** AGNT	Fourteen-Day, Repeat-Dose, Metabolism Study of Chloral Hydrate in Male and Female B6C3F1 Mice (CDER)	To establish the plasma levels of chloral hydrate and its metabolites in B6C3F1 mice.
E0210301 E0210311	**Beland** Contrera Dooley	Completed/ **Bio Tox/** AGNT	Fourteen-Day, Repeat-Dose, Range-Finding Study of Chloral Hydrate in Male and Female Fischer 344 (F344) Rats (CDER)	To determine the doses of chloral hydrate to be used in a chronic study.
E0210401 E0210411	**Beland** Contrera Dooley	Completed/ **Bio Tox/** AGNT	Fourteen-Day, Repeat-Dose, Metabolism Study of Chloral Hydrate in Male and Female Fischer 344 (F344) Rats (CDER)	To establish the plasma levels of chloral hydrate and its metabolites in Fischer 344 Rats.
E0210601	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Chronic Tumor Study of Fumonisin B1 in Male and Female B6C3F1 Mice (CFSAN)	To determine the tumorigenicity of fumonisin B1 in male and female B6C3F1 mice following chronic dietary exposure.
E0210801	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Chronic Tumor Study of Fumonisin B1 in Male and Female F344 Rats (CFSAN)	To determine the tumorigenicity of fumonisin B1 in male and female F344 rats following chronic dietary exposure.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0211001 E0211011 E0211012 E0211013	**Laborde** Bucci Collins Flynn Hansen Howard Shackleford Terry	Completed/ **Repro Lab, Bio Tox & Path/** AGNT	Developmental Toxicity Study of Fumonisin B_1 in Rabbits (CFSAN)	To determine the effect of Fumonisin B1 on rabbit development by administering the compound during organogenesis.
E0211101 E0211111 E0211121 E0211131 E0211141	**Howard** Binienda Casciano Couch Martinez Melchior Shaddock Slikker Sutherland Tolleson	Active/ **Bio Tox/** AGNT	The Role of Fumonisin B_1 in Fusarium sp. Tumorigenicity in Rats (CVM)	Determine the effect of fumonisin B1 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 subglu-tinans, Fusarium moniliforme* or a combination of the three fungi, using 32P-postlabeling technique. Determine the pharmacokinetics of fumon-isin B1 in B6C3F1 mice and F344 rats under conditions similar to those used in the chronic bioassay, and in non-human primates.
E0211301 E0211311 E0211321	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Sub-chronic (28-day) Study of Fumonisin B1 in Male and Female B6C3F1 Mice (CFSAN)	To determine the toxicity of fumonisin B1 in male and female B6C3F1 mice following a 28-day dietary exposure.
E0211401 E0211411	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Sub-chronic (28-day) Study of Fumonisin B1 in Male and Female F344 Rats (CFSAN)	To determine the toxicity of fumonisin B1 in male and female F344 rats following a 28-day dietary exposure.
E0211601	**Beland** Benson Contrera Gaylor	Active/ **Bio Tox/** AGNT	Tumorigenicity of Chloral Hydrate in B6C3F1 Mice (CDER)	To determine the effect of animal age and duration of exposure upon the tumorigenicity of chloral hydrate in female B6C3F1 mice.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0211701 E0211711 E0211722	**Leakey** Turturro Seng Contrera	Active/ **Chem Lab/** CNPT	Chronic Bioassay of Chloral Hydrate in Male B6C3F1 Mice Using Idealized Body Weight Curves that are Normalized by Modulation of Caloric Intake (CDER)	To determine the chronic toxicity and potential carcinogenicity of chloral hydrate, administered by aqueous gavage, to male B6C3F1 mice; To 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 ideal weight curve.
E0211801	**Culp** Mulligan Beland	Active/ **Bio Tox/** AGNT	Twenty-eight Day Range Finding Study in Mice and Rats Administered Malachite green or Leucomalachite Green in the Diet (CVM)	To 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 leu-comalachite green.
E0211901 E0211911	**Doerge** Rushing Churchwell Schmitt	Active/ **Chem Lab/** METH	Development of Analytical Methods for Determination of Malachite Green	1) Develop analytical methods to assess purity of malachite green (MG) and leucomalachite 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 condition.
E0212001 P00378	**Beland** Benson Chan Lorentzen Roberts	Active/ **Bio Tox/** AGNT	Effect of Ethanol on the Tumorigenicity of Urethane (Ethyl Carbamate) in B6C3F1 Mice (CFSAN)	To determine the effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0212101 P00367	**Doerge** Syvertson	Active/ **Chem Lab/** AGNT	Development of Analytical Methods for Determination of Urethane (CFSAN)	Develop analytical methods to assess purity and stability of urethane and ethanol that will be used as test compounds in the NTP rodent bioassay; 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; Develop analytical procedures to quantify the content of urethane in rodent feed.
E0212201 E0212211 E0212213 E0212214 E0212215	**Delclos** Newbold Weis Ferguson Germolec Ali Slikker	Active/ **Bio Tox & Neuro Tox /** AGNT	Range Finding Study for the Evaluation of the Toxicity of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats	To 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.
E0212301 E0212311 E0212313 E0212314 E0212315	**Delclos** Newbold Weis Germolec Ali Slikker	Active/ **Bio Tox & Neuro Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Methoxychlor Administered Feed to CD (Sprague-Dawley) Rats	To 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 Fumonison Derivatives in Female B6C3F1 Mice	Compare the toxicity of several fumonisin derivatives in female B6C3F1 mice.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0212501 E0212513 E0212514 X80009	**Delclos** Ferguson Scallet Newbold Weis Germolec Ali Meredith Rountree Slikker	Active/ **Bio Tox & Neuro Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Nonylphenol Administered in the Feed to CD (Sprague-Dawley) Rats	To determine whether pre/neonatal exposure to nonylphenol, a compound with estrogenic properties, will alter sex differences in behavior. The NTP has requested that data on the potential immunological effects of the test agents be collected during the range finding portion of the study.
E0212601 P00391 P00395 X80010	**Delclos** Doerge Newbold Weis Nestorick Schmitt Scallet	In Review/ **Bio Tox, Chem Lab & Neuro Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Vinclozolin Administered in the Feed to CD (Sprague-Dawley) Rats	To determine the doses of vinclozolin for use in a multi generation bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function. Also necessary to develop analytical methodology for vinclozolin in order to support the planned NTP rodent bioassays. Specific aims include 1) Develop a method to extract and quantify vinclozolin in rodent feed using electron-capture GC or other appropriate techniques; 2) Determine the stability and homogeneity of vinclozolin in rodent feed; and 3) determine purity and structurally characterize vinclozolin in test chemicals. A preliminary experiment will also be conducted in which pregnant dams will be fed vinclozolin at the high dose, 750 ppm in order to determine to what extent external feminization of the pubs will occur and if this will cause a problem in sex assignment.
P00374	**Delclos**	Completed/ **Bio Tox/** AGNT	Preparation of a Comprehensive Research Plan: Synthetic and Naturally Occurring Endocrine Disrupters	Overall research plan for the NTP/NCTR IAG to determine the effects of endocrine disrupting chemicals on fertility and reproductive tract cancers.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
P00392	**Doerge** Holder Siitonen	Active/ **Chem Lab/** METH	Development of Analytical Methods for Ethinyl Estradiol 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 Disrupter Study of the National Toxicology Program in conjunction with The National Center for Toxicological Research. As part of this study, the oral contraceptive agent ethinyl estradiol 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 conetne of etinyl estradiol in dosing medium. Because of the high potency of ethinyl estradiol, the challenge of this project will be coupling a low dosing level with the complex suite of coextractive compound found in rodents.
P00394	**Howard** Wamer	Active/ **Bio Tox/** AGNT	Development of a Research Plan for Testing the Carcinogenicity of A-Hydroxy Acids	Provide support for the development of a research plan and protocol for testing the carcinogenicity of the combination of α-hydroxy acids and UV light.
X60032	**Culp** Blankenship	Proposed/ **Bio Tox/** AGNT	2-Year Bioassay on Malachite Green	To be developed in FY-97.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
X6005101 X80011	**Delclos** Weis Ali Meredith Rountree Slikker	Proposed/ **Bio Tox & Neuro Tox/** AGNT	Multi-Generation Studies (Dose Range Finding Study - Ethynilestradiol	Project under development.
X60052	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (F0 Generation for Reproductive Assessment - Genistein)	Project under development..
X60054	**Delclos**	Proposed/ **Bio Tox**/ AGNT	Multi-Generation NTP Studies (F0 Generation for Cancer Assessment - Genistein)	Project under development..
X60056	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (F1 Generation for Cancer Assessment - Genistein)	Project under development..
X60057	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (F1 Generation for Reproductive Assessment - Genistein)	Project under development..
X60058	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F2 - Reproduction - Genistein	Project under development..
X60059	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F3 Gen - Reproduction - Genistein	Project under development..
X60060	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F3 Generation - Cancer - Genistein	Project under development..
X60061	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F4 Gen - Reproduction - Genistein	Project under development..
X60062	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F5 Generation - Reproduction - Genistein	Project under development..

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
X60072	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (F0 Generation for Reproductive Assessment - Methoxychlor	Project under development..
X60074	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Gen Studies - (F0 Gen - Cancer/Breeding - Methoxychlor	Project under development..
X60076	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F1 Generation for Cancer Assessment - Methoxychlor	Project under development..
X60077	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F1 Generation - Reproduction - Methoxychlor	Project under development..
X60078	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F2 - Reproduction - Methoxychlor	Project under development..
X60079	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F3 Gen - Reproduction - Methoxychlor	Project under development..
X60080	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F3 Generation - Cancer - Methoxychlor	Project under development..
X60081	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F4 Gen - Reproduction - Methoxychlor	Project under development..
X60082	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F5 Generation - Methoxychlor	Project under development..
X60092	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (F0 Generation for Reproductive Assessment - Nonylphenol	Project under development..
X60097	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F1 Generation - Reproduction - Nonylphenol	Project under development..
X60098	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F2 Reproduction - Nonylphenol	Project under development..

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
X60099	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F3 Gen - Reproduction - Nonylphenol	Project under development..
X60101	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F4 Gen - Reproduction - Nonylphenol	Project under development..
X60102	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F5 Generation - Reproduction - Nonylphenol	Project under development..
X60112	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F0 Gen - Reproductive Vinclozolin	Project under development..
X60117	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F1 Generation - Reproduction - Vinclozolin	Project under development..
X60118	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F2 Reproduction - Vinclozolin	Project under development..
X60119	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F3 Gen - Reproduction - Vinclozolin	Project under development..
X60121	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F4 Gen - Reproduction - Vinclozolin	Project under development..
X60122	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F0 Generation for Reproductive Assessment - Ethinylestradiol	Project under development..
X60127	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F1 Gen - Reproduction - Ethinyl Estradiol	Project under development..
X60128	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F2 Gen - Reproduction - Ethinyl Estradiol	Project under development..
X60129	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F3 Gen - Reproduction - Ethinyl Estradiol	Project under development..

Cooperating Organization: National Toxicology Program

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
X60131	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F4 Gen - Reproduction - Ethinyl Estradiol	Project under development..
X60132	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F5 Gen - Reproduction - Ethinyl Estradiol	Project under development..
X60133	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies - F5 Generation - Reproduction - Vinclozolin	Project under development..
X70011	**Howard**	Proposed/ **Bio Tox/** METH	DNA Adducts from the Pyrrolizidine Alkaloid Riddelline	Project under development..

COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENTS

COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENTS

Cooperating Organization: American Institute for Cancer Research

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0260101 E0260111 E0260112	**Lu** Djuric Hart Lewis	Active/ **Gen Lab/** CNPT	Modulation of Oxidative DNA Damage in Rats by Diet	1. To examine the relationships between the level of oxidative DNA damage and fat intake. 2. To examine the relationship between the level of oxidative DNA damage and energy intake.
E0260201 E0260211 E0260221	**Leakey** Gandy Manjgaladse Seng	Active/ **Chem Lab/** CNPT	Effect of Caloric Restriction on Rat Testicular Tumor Formation	All of the aims of this proposal are directed towards understanding the role of dietary components (i.e., caloric restriction) in influencing the ultimate susceptibility of the male reproductive tract to chemical insult.
E0260301 E0260311 E0260313 E0260321 E0260331	**Wolff** Kaput Visek	Active/ **Bio Tox/** CNPT	Caloric Restriction and Gene Expression in Agouti Mice (CDER)	The total amount of fat and calories we consume in our diet is highly correlated with the occurrence of cancer in North America and other highly developed nations. The studies proposed will help us learn how calories modify the development of cancer in mice and the mechanism underlying cancer development in humans.

Cooperating Organization: Electric Power Research Institute

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0672201 E0672202 E0672203 E0672223 E0672231 E0672233	**Beland** Culp Dooley Fullerton Kaderlik	Active/ **Bio Tox/** CNPT	Twenty-One Day Diet Range-finding Study	Develop methods for mixing coal tar residues in NIH-31 diets at various concentrations; Determine the pala-tability of a representative coal tar mixture that will be used in a subsequent chronic bioassay; Develop methods to quantify DNA adducts by 32P-postlabeling.

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0675200	**Cerniglia**	Active/ **Micro Lab/** CNPT	The Role of Human Intestinal Microflora in The Metabolism of Compounds in Gas Manufacturing Plant Residues	1. To determine the effect of compounds in gas manufacturing plant residues on the microbial activity and ecology of human intestinal microflora. 2. To determine the role of human intestinal microflora in metabolizing compounds contained in gas manufacturing plant residues.

Cooperating Organization: Gentest Corporation

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
X70054	**Leakey** Seng	Proposed/ **Chem Lab/** PRED	Predictive Systems for Human Drug Metabolism	Determine whether age, disease, diet and/or body mass influence express-ion 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: Astra Charwood

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0280001 P00387	**Paule** Binienda Gillam Hammond Pearson Slikker	Active/ **Neuro Tox/** PRED	Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions	1) To establish acquisition curves for several operant behaviors in juvenile rhesus monkeys during chronic oral exposure to two anticonvulsant agents and vehicles; 2) To determine whether such exposure results in any significant changes in the acquisition and performance of these operant and other observable behaviors; 3) To determine whether such exposure results in any significant changes in clinical chemistry or ophthalmic parameters; 4) To determine plasma distribution profiles and concentrations for each of these agents at various stages of chronic exposure.

1997 NCTR PUBLICATIONS

1997 NCTR Publications

The following list of NCTR publications includes those publications which were accepted for publication or published during the calendar year, 1997 (1996 publications are included and indicated as such, if they were not printed in "NCTR's Research Plans and Accomplishments, 1996-1997).

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 show below:

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

Division/Contractor Abbreviations:

Biochemical Toxicology (Bio Tox)
Biometry & Risk Assessment (Biometry)
Division of Facilities, Engineering Management (DFEM)
Genetic & Reproductive Toxicology (Gen & Repro)
Microbiology & Chemistry (Micro & Chem)
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)

1997 NCTR Publications

1. Acchiardo, S.R., Moore, L.W., Feuers, R.J., Dulaney, J.T., Burk, L.B., Smith, S.O. and Cardoso, S.S. Activity of antioxidant enzymes in red blood cells of hemodialysis patients. Journal of Renal Nutrition and Metabolism, Accepted: 6/05/97. **(E0699101) (PREDICTION OF TOXICITY) (Gen & Repro)**

2. Ahn, H. and Chen, J.J. A two-way analysis of covariance models for classification of stability data. Biometrical J., 39(4):559-576. **(E0690901) (METHOD-DRIVEN) (Biometry)**

3. Ahn, H. and Kodell, R.L. Analysis of long-term carcinogenicity studies. Design and Analysis of Animal Studies in Pharmaceutical Development, Accepted: 6/1/97. **(PREDICTION OF TOXICITY) (Biometry)**

4. Aidoo, A., Morris, S.M. and Casciano, D.A. Development and utilization of the rat lymphocyte *hprt* mutation assay. Mutation Research Reviews in Mutation Research, 387:69-88. **(E0697501) (PREDICTION OF TOXICITY) (Gen & Repro)**

5. Ali, S.F., French, E.D. and Dillon, K. Effects of ibogaine, and cocaine and morphine after ibogaine, on ventral tegmental doipamine neurons. Pharmacology Letters, Accepted: 10/15/96. **(AGENT-DRIVEN) (Neuro Tox)**

6. Ali, S.F., Newport, G.D., Rothman, R.B., Slikker, W., Jr. and Baumann, M.H. Neuroendocrine and neurochemical effects of acute ibogaine administration: A time course evaluation. Brain Research, Accepted: 10/15/96. **(AGENT-DRIVEN) (Neuro Tox)**

7. Ali, S.F., Newport, G.D. and Slikker, W., Jr. Methamphetamine-induced dopaminergic toxicity in mice: role of environmental temperature and pharmacological agents. Annals of the New York Academy of Sciences, Accepted: 10/31/96. **(AGENT-DRIVEN) (Neuro Tox)**

8. Allen, L.B., Siitonen, P.H. and Thompson, H.C. Determination of copper, lead, and nickel in edible oils by plasma and furnace atomic spectroscopies. J. American Oil Chemist's Society, Accepted: 10/14/97. **(E0689201) (METHOD-DRIVEN) (Micro & Chem)**

9. Allen, L.B., Siitonen, P.H. and Thompson, H.C. Methods for the determination of arsenic, cadmium, copper, lead and tin in sucrose, corn syrups and high fructose corn syrups by inductively coupled plasma atomic emission spectrometry. Journal of Agricultural and Food Chemistry, 2:1. **(E0698201) (METHOD-DRIVEN) (Micro & Chem)**

10. Allen, P.T. and Poirier, L.A. Suppression by phenobarbital of ethionine-induced hepatocellular carcinoma formation and hepatic S-adenosylethionine levels. Carcinogenesis, 18(5):1103-1107. **(CONCEPT-DRIVEN) (Mol Epi)**

11. Ambrosone, C.B., Freudenheim, J.L., Graham, S., Sinha, R., Marshall, J.R., Vena, J.E., Laughlin, R., Nemoto, T., Gillenwater, K.A., Harrington, A.M. and Shields, P.G. Breast cancer risk, meat consumption and metabolism of food-derived heterocyclic amines by N-acetyltransferase. International Journal of Cancer, Accepted: 9/01/97. **(AGENT-DRIVEN) (Mol Epi)**

12. Ambrosone, C.B. Environmental Organochlorine Exposure and Postmenopausal Breast Cancer Risk. J. National Cancer Institute, Accepted: 5/15/97. **(PREDICTION OF TOXICITY) (Mol Epi)**

13. Ambrosone, C.B. and Kadlubar, F.F. Towards an integrated approach to molecular epidemiology. American Journal of Epidemiology, 146:1-7. **(CONCEPT-DRIVEN) (Mol Epi)**

14. Ambrosone, C.B. and Kadlubar, F.F. Acetyltransferase polymorphisms and disease susceptibility. In: Biomarkers, the Genome, and the Individual, (Joseph Henry Press; NAS) In Press. **(PREDICTION OF TOXICITY) (Mol Epi)**

15. Ambrosone, C.B. and Kadlubar, F.F. Meeting Report: Formation of a molecular epidemiology group? Cancer Epidemiology, Biomarkers and Prevention, 6:651-653. **(CONCEPT-DRIVEN) (Mol Epi)**

16. Anderson, K., Hammons, G.J., Ilett, K., Kadlubar, F.F., Potter, J.D., Kaderlik, K., Minchin, R.F., Teitel, C.H., Chou, H., Guengerich, F.P. and Lang, N.P. Metabolic activation of aromatic amines by human pancreas. Carcinogenesis, 18(5):1085-92. **(E0694601) (PREDICTION OF TOXICITY) (Mol Epi)**

17. Ang, C.Y., Luo, W., Kiessling, C.R., Mckim, K.L., Lochmann, R., Walker, C.C. and Thompson, H.C. A bridging study between liquid chromatography and microbial inhibition assay methods for determination of amoxicillin residues in catfish muscle. Journal of AOAC International, Accepted: 8/19/97. **(E0693601) (METHOD-DRIVEN) (Micro & Chem)**

18. Ang, C.Y. and Luo, W. Rapid determination of ampicillin in bovine milk by liquid chromatography with fluorescence detection. Journal of AOAC International, 80(1):25-30. **(E0693601) (METHOD-DRIVEN) (Micro & Chem)**

19. Ang, C.Y., Luo, W., Call, R. and Righter, H.F. Comparison of liquid chromatography with microbial inhibition assay for determination of incurred amoxicillin and ampicillin residues in milk. J. Agri. Food Chem., 45(11):4351-4356. **(E0693601) (METHOD-DRIVEN) (Micro & Chem)**

20. Arani, R.B. A result on a 2x2 survival experiment. Mathematical Biosciences, Accepted: 5/21/97. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

21. Arani, R.B. and Chen, J.J. A sequential method of p-value adjustment for correlated endpoint. Proceedings 1997 ASA Joint Statistical Meetings, Accepted: 8/13/97. **(E0700901) (METHOD-DRIVEN) (Biometry)**

22. Baker, M.E., Medlock, K.L. and Sheehan, D.M. Flavonoids inhibit estrogen binding to rat alpha-fetoprotein. Proc. Soc Exp Biol, Accepted: 6/12/97. **(CONCEPT-DRIVEN) (Gen & Repro)** (Micro)

23. Banfalvi, G., Poirier, L.A., Mikhailova, M.V. and Chou, M.W. Relationship of repair and replicative DNA synthesis to cell cycle in chinese hamster ovary (CHOK1) cells. DNA and Cell Biology, 16:1155-1160. **(E0696701) (AGENT-DRIVEN) (Bio Tox)** (Mol Epi)

24. Banfalvi, G., Mikhailova, M.V., Poirier, L.A. and Chou, M.W. Multiple subphases of DNA replication in CHO cells. DNA and Cell Biology, Accepted: 4/01/97. **(E0696701) (AGENT-DRIVEN) (Bio Tox)** (Mol Epi)

25. 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. J. Tox. Environ. Health, Accepted: 11/02/97. **(E0210101) (AGENT-DRIVEN) (Bio Tox)** (Biometry) (Micro & Chem)

26. Beland, F.A., Melchior, W.B., Mourato, L.M., Santos, M. and Marques, M.M. Arylamine-DNA adduct conformation in relation to mutagenesis. Mutation Research, 376:13-19. **(E0673700) (PREDICTION OF TOXICITY) (Bio Tox)**

27. Benson, R.W. and Beland, F.A. Modulation of urethane (ethyl carbamate) carcinogenicity by ethyl alcohol: A Review. Int. J. Toxicol, 16:521-544. **(E0212001) (AGENT-DRIVEN) (Bio Tox)**

28. Bezalel, L., Hadar, Y. and Cerniglia, C.E. Enzymatic mechanisms involved in phenanthrene degradation by the white rot fungus *Pleurotus ostreatus*. Appl. Environ. Microbiology, 63:2495-2501. **(AGENT-DRIVEN) (Micro & Chem)**

29. Binienda, Z.K. Compensatory long-term effect of perinatal hypoxia-ischemia: A possible mechanism for neuroprotection? Annals of the New York Academy of Sciences, 825:146-151. **(E0692301) (AGENT-DRIVEN) (Neuro Tox)**

30. Binienda, Z.K. and Kim, C. Increase in levels of total free fatty acids in rat brain regions following 3-nitropropionic acid administration. Neuroscience Letters, 230:199-201. **(E0692301) (AGENT-DRIVEN) (Neuro Tox)**

31. Bolon, B., Bucci, T.J., Warbritton, A.R., Chen, J.J., Mattison, D.R. and Heindel, J.J. Follicle differential counts as a screen for ovarian toxicity in rodents: Results from continuous breeding bioassays. Fundamenta Applied Toxicology, Accepted: 6/23/97. **(E0045800) (Research) (Pathology)** (Biometry)

32. Cerniglia, C.E. Fungal metabolism of polycyclic aromatic hydrocarbons: Past, present and future applications in bioremediation. Journal of Industrial Microbiology, 19:324-333. **(AGENT-DRIVEN) (Micro & Chem)**

33. Chae, Y.H., Upadhyaya, P., Fu, P.P., El-Bayoumy, K. and Ji, B. Comparative metabolism and DNA binding of 1-, 2-, and 4-nitropyrene in rats. Mutation Research, 376(1-2):21-28. **(E0657300) (PREDICTION OF TOXICITY) (Bio Tox)**

34. Chen, J.J. and Tsong, Y. Multiple time-point dissolution specifications for sampling acceptance plan. Journal of Biopharmaceutical Statistics, 7(2):259-270. **(S00009) (CONCEPT-DRIVEN) (Biometry)**

35. Chen, T., Mittelstaedt, R.A. and Heflich, R.H. DNA sequence flanking the protein coding regions of the rat *hprt* gene. Mutation Research Genomics (Annotated Sequence), Accepted: 9/25/97. **(E0695801) (PREDICTION OF TOXICITY) (Gen & Repro)**

36. Chen, J.J. Analysis of reproductive and developmental studies. Design and Analysis of Animal Studies in Pharmaceutical Development, Accepted: 6/01/97. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

37. Chen, T., Aidoo, A., Casciano, D.A. and Heflich, R.H. Expansion of rat 6-thioguanine-resistant T-lymphocyte clones by stimulation with ionomycin and phorbol ester. Environmental and Molecular Mutagenesis, Accepted: 8/03/97. **(E0695801) (PREDICTION OF TOXICITY) (Gen & Repro)**

38. Chen, J.J. and Ahn, H. Marginal models with multiplicative variance components for over-dispersed binomial data. Journal of Agricultural, Biological and Environmental Statistics, Accepted: 9/02/97. **(E0684300) (METHOD-DRIVEN) (Biometry)**

39. Chen, J.J., Kodell, R.L. and Pearce, B.A. Significance levels of randomization trend tests in the event of rare occurrences. Biometrical Journal, 39(3):327-337. **(E0690201) (PREDICTION OF TOXICITY) (Biometry)**

40. Chen, J.J., Tsong, Y. and Ahn, H. Shelf-life estimation for multifactor stability studies. Drug Information Journal, 31(2):573-587. **(E0690901) (METHOD-DRIVEN) (Biometry)**

41. Chiarelli, M.P. and Lay, J.O. MALDI TOF MS: Food related applications. In: Spectral Methods in Food Analysis, In Press. **(METHOD-DRIVEN) (Micro & Chem)**

42. Chou, M.W., Chen, W., Mikhailova, M.V., Nichols, J.A., Weis, C.C., Jackson, C.D., Hart, R.W. and Chung, K. Dietary restriction modulated carcinogen-DNA adduct formation and the carcinogen-induced DNA strand breaks. Tox Lett, 92:21-30. **(E0260401) (CONCEPT-DRIVEN) (Bio Tox)** (OD/Imm Off)

43. Chou, H., Ozawa, S., Fu, P.P., Lang, N.P. and Kadlubar, F.F. Metabolic activation of methylhydroxylated derivatives of 7,12-dimethylbenz[a]anathracene by human liver dehydroepiandrosterone-steroid sulfotransferase. Carcinogenesis, Accepted: 8/02/97. **(METHODS-DRIVEN) (Mol Epi)** (Bio Tox)

44. Chou, M.W. and Chen, W. Food restriction reduces AFB_1-DNA adduct formation AFB_1-glutathione conjugation, and DNA damage in AFB_1-treated male F344 rats and B6C3F1 mice. Journal of Nutrition, 127(2):210-217. **(E0260401) (CONCEPT-DRIVEN) (Bio Tox)**

45. Churchwell, M.I., Cooper, W.M., Howard, P., and Doerge, D.R. Determination of fumonisins in rodent feed using HPLC with electrospray mass spectrometric detection. J. Agric. Fd. Chem., 45(7):2573-2578. **(S00007) (METHOD-DRIVEN) (Micro & Chem)** (Bio Tox)

46. Clausing, P.P., Rushing, L.G., Newport, G.D. and Bowyer, J.F. Determination of d-fenfluramine, d-norfenfluramine and fluoxetine in plasma, brain tissue and brain microdialysate using high-performance liquid chromatography after precolumn derivatization with dansyl-chloride. J. Chromatography B: Biomedical Applications, 692(2):419-26. **(E0690301) (CONCEPT-DRIVEN) (Neuro Tox)** (Micro & Chem)

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