NCTR: A Look Back

Established by executive order in 1971, The National Center for Toxicological Research (NCTR) is internationally recognized for research that addresses the mechanisms of toxicity of chemicals and pharmaceutical drugs; the risks associated with chemical and microbial food contamination, and identifies biomarkers for terrorism due to biological and/or chemical exposure.

1971
Established principles of operation, management, and administration of NCTR. Environmental Protection Agency (EPA) became a funding member of the Center.

Establishment of Scientific Advisory Board to advise the Director, NCTR, in establishing, implementing, and evaluating the research programs that assist the Commissioner of Food and Drugs in fulfilling his regulatory responsibilities. The Board provides an extra-agency review in ensuring that the research programs at NCTR are scientifically sound and pertinent.

1971-2
Developed design criteria for a "Barrier System" (a specific pathogen-free animal holding facility) and criteria for chemical compound(s) and animal strain(s) selection.

1972
Initiated the Interdisciplinary Toxicology Program (INTOX) with the University of Arkansas for Medical Sciences (UAMS) for graduate studies leading to a doctoral degree in toxicology. Detected bacterial pathogens in the cage water from commercially purchased mice.

1974

Began the ED01 Study (Effective Dose 1 out of 100) upon completion of the "Barrier System," on **April 15, 1974**, by loading the first mouse (EDIE) onto an experiment that would involve 24,000+ mice.

1973-5
Completed integrated computer data systems for animal breeding **(1973)**, allocation **(1974)**, experimental data collection **(1974)**, pathology **(1975),** and microbiological, chemical, and environmental monitoring systems. **(1975**)

1975
Initiated the Interagency Agreement (IAG) with John H. McClellan Veterans Administration Medical Center to support collaborative projects and postdoctoral training program.

1976
Conducted formal training for FDA inspectors on aspects of Good Laboratory Practices (GLP) in Little Rock area.

1977
Received AAALAC (American Association for Accreditation of Laboratory Animal Care) accreditation for laboratory animal facilities and practices--a first for an FDA laboratory.
Selected Employer of the Year by the State of Arkansas for its employment opportunities for mentally/emotionally handicapped young adults.

1978
Committed one-third of its resources to support the National Toxicology Program (NTP) which was established in 1977. NTP contracted with NCTR for the design, development, and implementation of an Automated Toxicology Data Management System (TDMS).

1979
Completed and occupied the Teratology Laboratories (Bldg. 53) and Multispecies Breeding Facility.

Completed the ED01 Study when the last remaining laboratory animal (ENDIE) was removed (1977) and results of the study were presented at a symposium in Washington, D.C.

1980
Proposed that bulky carcinogen-DNA adduct would give rise to transversion mutations in mammalian genes, including protooncogenes, a hypothesis that is now widely supported by numerous investigations.
Determined biochemical pathways for the degradation of toxic chemicals, using state-of-the-art analytical chemistry techniques.

Established and maintains the only primate colony within FDA for use in studies on risk assessment for drug/chemical exposures. The primate colony provides a unique resource for addressing issues of species extrapolation as it relates to chemical markers of exposure (tissue levels, pharmaco/toxicokinetics, and metabolism). The nonhuman primate model is particularly

powerful for providing data on the transplacental movement of drugs (after maternal exposures) into the primate fetus, and thus metrics of *in utero* fetal exposure.

Used microorganisms as biocatalysts to degrade and mineralize toxic chemicals during bioremediation and to catalyze the synthesis of useful compounds, such as agrichemicals, pharmaceuticals, flavors, fragrances, and other fine chemicals by biotransformation.

Detected pathogenic endoparasites and pathogenic bacteria in Rhesus monkeys received from a commercial vendor. Surveillance/Diagnostic efforts prevented these animals from being introduced into the NCTR primate colony until they had been treated.

Isolated microorganisms involved in the biodegradation of pollutants and related compounds. Developed microcosm test systems to determine pathways and evaluated detoxification rates for priority environmental pollutants.

1980-5
Assisted in the creation of the Arkansas Science and Technology Authority permitting a closer research collaboration with universities of the region.

Expanding Interactions: Emphasis on peer-reviewed research and publication of research manuscripts and final reports.

Developed the first direct proof that DNA damage was causal in UV-induced carcinogenesis.

Participated in the development and editing of the White House Office of Science and Technology Policy Document on Chemical Carcinogenesis, which led to the first formulation of the default assumptions used in the risk assessment of chemical carcinogens, including the assumption that chemically induced toxicity was "independent of diet."

Participated in the development and editing of the White House Document on Formaldehyde leading to a revision of the formaldehyde regulations.

Addressed, at the request of the Assistant Secretary of Health and Commissioner of the FDA, the issue of "de minimus" through an objective scientific evaluation of the risks posed by selected food dyes.

1980-90
Contributed to the understanding of inter-individual differences in human cancer susceptibility through the characterization of the major drug- and carcinogen-metabolizing enzymes involved in both bioactivation and detoxification. These included the first human studies on the enzymatic *O*-acetylation, *O*-sulfonylation, and *N*-glucuronidation of proximate carcinogenic metabolites of environmental and foodborne aromatic and heterocylic amines.

1980-95
Defined the metabolic pathways and target tissue specificities for several known and probable human carcinogens, including 4-aminobiphenyl, 2-naphthylamine, benzidine, methylene-*bis*(2-

chloroaniline), 2-amino 1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), and 2-amino-3-methylimidazo(4-5-f)quinoxaline(MelQx) through comparative *in vitro* and *in vivo* studies in experimental animals and humans.

1981
Hosted meeting of the National Academy of Sciences (NAS) Board on Toxicology and Environmental Health Hazards.

Completed construction of a quarantine facility for purchased laboratory animals and an electron microscope facility.

1982
EPA terminated funding support.

1983
Examined how exogenous compounds, such as antimicrobial residues, probiotics, and food additives affect the intestinal microflora, including changes in bacterial populations, antimicrobial resistance, and colonization-barrier effects.

Used traditional culture methods, biochemical techniques, molecular-based methods, membrane arrays, and microarray methods to detect human intestinal microflora.

1984
Completed a solar power demonstration project and an interim diet preparation facility.

1985
Initiated IAGs with other government agencies for collaborative research (i.e., Department of Defense, National Institute for Environmental Health Sciences, National Institute on Drug Abuse, National Institute on Aging, Consumer Products Safety Commission, National Institute for Dental Health).

Established and maintained clinical collaborations with the Arkansas Cancer Research Center (University of Arkansas for Medical Sciences) and the Central Arkansas Veterans Healthcare System that have provided a direct interface between NCTR research and its extrapolation to humans.

Negotiated an IAG with the Department of Energy (DOE) through the auspices of the Oak Ridge Associated Universities (ORAU) to provide student and postdoctoral appointees practical research training opportunities at NCTR.

1985-90
Participated in drafting language vital to adoption of the Federal Technology Transfer Act of 1986.

1986

Demonstrated the utility of the nonhuman primate as an outstanding model for pediatric/developmental drug studies: (*in utero* production of cocaine babies,; anticonvulsant assessments in juveniles; marijuana smoke exposure during adolescence). The ability of nonhuman primates to perform cognitive tasks (learning, memory, time perception, visual discrimination, etc.) with direct applicability to human pediatric populations have provided the opportunity for direct interspecies comparisons in complex brain function.

Used an *in vitro* culture system which simulates the human colonic environment to determine changes to the normal intestinal microflora due to dietary exposure to food contaminants.

1987
Completed and dedicated new laboratories/animal rooms in Building 53 with Triad Celebration.

1988
Completed the first Summer Student Research Program.

Accepted research manuscripts fall within the top 25% of peer-reviewed journals world-wide as measured by journal impact factor found in the Science Citation Index.

Signed collaborative research agreements with the Republic of South Korea, the Republic of China (Taiwan), and a major medical school in the People's Republic of China.

1989
Hosted a Toxic Waste Workshop involving emerging scientific leaders from the United States and the Soviet Union.

Developed and applied dose-response models for probabilistic risk assessment of microbial illness, cancer, developmental defects, and continuous measures of toxicity. This included developing mathematical models based on postulated biological mechanisms, fitting the models to data on toxic substances to estimate risks and/or benchmark doses, and developing model averaging techniques to account for model uncertainty. This work included seminal contributions to the so-called “hybrid” approach for calculating benchmark doses for continuous toxic effects, which has become the preferred regulatory approach. Work continues on a hierarchical linkage of PK and PD models and on a mathematical structure for naturally propagating inter- and intra-species uncertainties.

Established complimentary human laboratories for directly comparing human and animal cognitive performance utilizing identical behavioral tasks. Such efforts help to establish relevant measures for assessing drug effects in animal models for cross-species extrapolation and greatly assist the risk assessment process. In addition, it has been demonstrated that the behavioral instrument used (the NCTR Operant Test Battery) is sensitive to clinical entities such as Attention Deficit Hyperactivity Disorder; depression; anxiety, Alzheimer's Disease, and dizziness.

1989-2004

Developed statistical theory and methods for assessing risks of chemical mixtures. This involved developing experimental designs for testing chemical mixtures for additivity, synergism, and antagonism; and developing statistical algorithms for determining classes of chemicals in mixtures having similar dose-responses, for assessing the cumulative risk and for setting exposure limits. This work, much of which was done in collaboration with scientists at EPA under an interagency agreement for developing relative potency factors for pesticide mixtures, led to an EPA bronze medal for commendable service.

1990
Participated in the Edwards Advisory Committee on the Food and Drug Administration (Blue Ribbon Panel) review of FDA.

Developed metabolic phenotyping, genotyping, and sequencing methods for assessing the expression of drug and carcinogen metabolism and DNA repair enzymes in humans. Together with epidemiological data, established a model which indicates that susceptibility factors and dietary exposures to foodborne heterocyclic amines could account for nearly half of the sporadic colo-rectal cancer incidence in the U.S.

Improved and validated methodologies for the identification of carcinogen-DNA adducts in humans and provided the first evidence for the occurrence of polycyclic aromatic hydrocarbons, and of aromatic and heterocyclic amine adducts in human carcinogen-target tissues, including the urinary bladder, larynx, pancreas, lung, colon, breast, and prostate.

1990-1996
Participated in the development and establishment of the Arkansas Science and Math High School.

1991
Focused research studies to have more relevance to FDA's needs with scientists from NCTR visiting FDA centers in Washington to improve research integration.

Implemented an Activity-Based Cost Accounting Project Management System at the Center.

1992
Established an Interagency Agreement (IAG) with the National Institute for Environmental Health Sciences (NIEHS) that provides funding for research and testing of chemicals and agents nominated by FDA to the National Toxicology Program (NTP).

1993
Provided Special Employment Programs (temporary appointments), which included as many as 245 students, postgraduates, and guest workers on-site at the NCTR, most of whom were engaged in research.

Added a foreign national presence at NCTR with appointees representing as many as 17 different countries in any given year.

Hosted an international group of inspectors interested in Biological Weapons Treaty Issues.

1994
Participated in an FDA-wide evaluation of Food Related Research.

Established cell culture and electrophysiological capabilities and developed new economical staining techniques (cell death; myelin) to enhance analytical capabilities.

Developed rapid and sensitive detection methods (conventional and molecular) and advanced existing methodologies for isolating and identifying microbial agents and toxins in fresh and processed foods.

Developed, validated and improved commercial test kits used for the detection of foodborne pathogens and toxins in complex food matrices.

1995
Assisted the Office of Regulatory Affairs (ORA) on laboratory consolidations and planning for the establishment of the Jefferson Regional Laboratories.

At the forefront of discovery of novel biomarkers of detection, treatment, and prevention of pancreatic cancer including *NF-kappa B*, *STAT3*, **NQO1**, and *MnSOD*, as well as on the role of DNA methylation of the *k-ras* gene by phytoestrogens.

Developed method for recycling waste ethanol, methanol, acetone, xylene to histological grade solvents, and waste formalin to 10% neutral buffered formalin resulting in significant cost savings for purchase and disposal of reagents.

Developed method to prepare tissues for the evaluation of sexually dimorphic nuclei in the brain of rats. Included development of procedure to produce a flat surface; digital imaging of brains, and outlining specific nuclei and other anatomical reference points of the brains to facilitate two dimensional and three dimensional analyses of the sexually dimorphic nuclei.

Purchase, set-up and implementation of Hamilton-Thorne Integrated Visual Optical System (IVOS) to analyze rat sperm samples for motility at NCTR to enable the following procedures: automated sperm motility, automated sperm counts, sperm morphology, capability of saving digital image of motility and counts. (1998-present). Image analysis capability using PC-based Optimus and Image Pro Plus image analysis software.

Image analysis capability using PC based Optimus and Image Pro Plus image analysis software.

Developed immunostaining protocol for: histone1 and beta-catenin, and histone1 and beta-actin, 11β-hydroxysteroid dehydrogenase (marker for Leydig cells) polycystin-2, double immunofluorescent staining for histone H1 and β-catenin, Melan A, TRP-1, anti-fibroblast, pan-cytokeratin plus, p8, IRS-3,IRS-4, fatty acid, synthase, apoA-IV, trimethyl-histone H4 (Lys20), anti-mouse Ki-67 (clone TEC 3), iNOS, nitrotyrosine, CD48, CD55, validation of *in situ* hybridization for histone mRNA and MIB-5 immunohistochemistry (IHC) as proliferation assays

that can substitute for BrdU IHC when necessary, detection of apoptotic cells with caspase-3 in rat adrenal gland. Other IHC protocols developed at the request of the investigator.

Developed a program for laboratory technicians accepted and approved by the U.S. Department of Labor as apprenticeship training for the Journeyperson position.

Developed a system for capturing cost of supplies and labor for IAG and non-IAG studies, which provides a mechanism for monitoring projected versus actual costs and provides documentation for "billing" IAG program costs

1995-7
Determined DNA adduct levels of the human carcinogen 4-aminobiphenyl in human peripheral lung in relation to metabolic activation pathways involving pulmonary *N*-oxidation, conjugation, and peroxidation.

1995-9
At the request of the Center for Food Safety and Applied Nutrition, investigated the carcinogenicity of fumonisin B_1, a toxin produced by fungi that grow on corn and other plants worldwide. Under the conditions of the bioassays, fumonisin B_1 was demonstrated to be a liver carcinogen in female mice and renal carcinogen in male rats. These studies formed the basis for setting acceptable levels of fumonisin in food worldwide. In addition, demonstrated that fumonisin B_1 mechanism of action is induction of apoptosis in liver and kidney cells. Established that programmed cell death results from ceramide depletion as well as sphinganine accumulation in human cells treated with the mycotoxin fumonisin B_1.

Determined the relative importance of cytochrone P450 2E1 and 1A2 in the pathogenesis of acetaminophen-induced hepatotoxicity in mice. Determined the effects of acetaminophen metabolism on cytochrome P450 2E1 and 1A2 function. Determined that the protection afforded by repeat exposure to acetaminophen is the result of localized destruction of centrilobular-localized cytochrome P450 2E1 and subsequent bioactivation of acetaminophen by other enzymes in the periportal region where detoxification and repair mechanisms are more efficient.

1995-2000
To assist in making drug regulatory decisions by the FDA, designed and conducted studies to establish the neonatal mouse tumorigenicity bioassay as an alternative tumorigenicity bioassay. At the request of the Center for Drug Evaluation and Research, investigated the carcinogenicity of chloral hydrate, a sedative used in pediatric medicine. Under the conditions of the bioassay, there was equivocal evidence of carcinogenic activity.

Established that while neonatal mouse tumorigenicity bioassay is highly sensitive to carcinogens that induce tumors through exogenous DNA adduct formation, it is not sensitive to carcinogens that induce tumors through other mechanistic pathways.

1996
Constructed a new Library/Conference Center and obtained funding for the construction of an NCTR/ORA Quarantine Facility**.**

Commemorated its 25th Anniversary with two events: Dedication of New Library and ground breaking for ORA facility; and FDA Science Day.

1996-1999
Developed methodology for the quantitative detection of the mycotoxins fumonisin B_1, B_2, and B_3, and hydrolyzed fumonisin B_1 in corn-based feed using automated on-line immunoaffinity chromatography coupled with high performance liquid chromatography and mass spectrometry. Conducted studies to elucidate the interactive effects of methyl-deficiency and dietary restriction on liver tumors induced by the mycotoxin aflatoxin B_1.

1996-2000
Conducted collaborative studies with the Center for Food Safety and Applied Nutrition to assess cancer risk posed by intermittent exposures to aflatoxin B_1 in rats.

1996-2003
At the request of the Center for Food Safety and Applied Nutrition, investigated the carcinogenicity of urethane, a by-product of fermentation, in the present of alcohol. Under the conditions of the bioassay, urethane induced cancer at several sites, and there was weak evidence that ethanol affected the carcinogenicity of urethane.

1996-2004
Developed sensitive liquid chromatography/mass spectrometry methods for analysis of β-adrenergic growth promoters in livestock tissues.

1996-2005
Participated on Environmental Protection Agency and National Institute for Environmental Health Sciences panels concerned with the development and evaluation of screens and tests for compounds with endocrine modulating activities.

Developed and validated numerous analytical chemical methods of analysis to determine concentrations of test substances in dosage forms in support of National Toxicology Program studies.

1996-present
Characterized proteins and clone genes for the important enzymes from microorganisms that degrade toxic chemicals.

1997
Founded the Molecular Epidemiology Group of the American Association for Cancer Research.

1997-2000
Identified isoflavones, genistein, and daidzein, as the antithyroid constituents in soy.

Determined the tumorigenicity of benzodiazepines, antihistamines, chloral hydrate, tamoxifen, hydroxyzine, methylphenidate, clofibrate, toremifene, and proton pump inhibitors by the neonatal mouse tumorigenicity bioassay.

1997-2003
Developed and validated methods of analysis for determination of ethinyl estradiol in rodent diets. Studies sponsored by the National Toxicology Program on ethinyl estradiol required methods with extreme sensitivity to determine ethinyl estradiol at a low dose level of 1 µg/kg.

1997-2003
Developed and validated a liquid chromatographic method to determine fourteen sulfonamides in catfish, salmon and shrimp at the 10 µg/kg level ????. The project was designed to provide a regulatory method to ensure consumer safety from consumption of sulfonamides in aquacultured species.

1997-2005
In conjunction with the National Toxicology Program, conducted a series of studies designed to evaluate aspects of the endocrine disruptor hypothesis, that is, that hormonally active agents present at low levels in the environment are adversely affecting human health, including reproductive and immune function and cancer. Test compounds included the soy isoflavone genistein, the industrial intermediate nonylphenol, and the potential estrogen ethyinyl estradiol. Investigated the mechanisms by which tamoxifen, an adjuvant chemotherapeutic agent, induces liver cancer in rodents. Developed sensitive liquid chromatography/mass spectrometry methods for the detection of tamoxifen DNA adducts and applied these techniques to women being treated with tamoxifen.

1998
Developed sensitive liquid chromatography/mass spectrometry methods for analysis of malachite green, a carcinogenic dye used illegally in aquaculture, and metabolites in fish and rodent tissues.

1998-2001
Developed methodology for the quantitative analysis of the oxidative DNA adduct etheno-2'-deoxycytidine DNA using on-line immunoaffinity chromatography coupled with liquid chromatography coupled with electrospray tandem mass spectrometry detection.

1998-2002
Developed methodology for the simultaneous quantitation of 12 estrogen metabolites based on on-line solid phase extraction with coulometric detection. Used this methodology to elucidate the effects of cetechol-*O*-methyl tranferase inhibition on estrogen metabolism and oxidative DNA damage levels in estradiol treated MCF-7 cells.

1998-2003
Analyzed methylmercury disposition in humans utilizing a PBPK model and animal (12 species) pharmacokinetic data. The resulting human model, in accord with the animal models, predicted relatively high inorganic mercury levels in the kidneys long after the disappearance of

methylmercury from the blood. This work represents one of the most comprehensive studies on the use of PBPK modeling for interspecies extrapolation in risk assessment.

1998-2005
At the request of the Center for Veterinary Medicine, investigated the carcinogenicity of malachite green, a dye used to prevent fungal infections in commercial fisheries. Under the conditions of the bioassay, leucomalachite green, a metabolite of malachite green that is found in edible tissue, was demonstrated to induce liver caner in mice.

1998-present
Isolated, characterized and identified, using cultural and molecular methods, pathogens from processed foods, aquaculture, clinical, and environmental samples. **(**
Investigated xenobiotic metabolism, such as that of phytoestrogens, food additives, and supplements, by intestinal microflora and determined the impact these substances have on the microflora in the human gastrointestinal tract.

Conducted antimicrobial susceptibility testing of bacterial isolates using standardized methods, including Sensititre, micro-broth dilution assays, disk-diffusion assays, and E-tests.

1999-2005
Developed statistical methods to identify photocarcinogenesis risks. This included developing a biologically based structural model for separating effects on tumor frequency from effects on tumor latency, and applying the model to photocarcinogenesis data on transgenic animals to characterize risks from cosmetics and other skin products. This methodology allows regulators to differentiate between initiating effects and promoting effects of products that may interact with sunlight to produce skin cancer.

1999-present
Developed improved survival-adjusted/age-adjusted statistical tests and novel estimators of tumor progression time for data from long-term tumorigenicity bioassays for hazard identification. This included developing statistical methods for attributing tumor lethality when cause of death is not assigned in rodent bioassays; developing a constrained nonparametric maximum likelihood estimation method for time to onset of occult tumors in the absence of cause-of-death information; developing an improved survival-adjusted test robust to various underlying tumor onset distributions; and developing a probabilistic procedure for estimating the lag time between the onset of and death from an occult tumor using the imputed cause-of-death information. Research on improved statistical methods for assessing the potential carcinogenicity of FDA-regulated products continues.

Developed models and data for the transmission dynamics of microbial pathogens. This involved developing novel models for disease spread based on sensitive sub-populations, conducting animal experiments to acquire data for characterizing sensitivities for infection and illness, and validating the models by mimicking epidemiology data on actual outbreaks. This work, funded by an interagency agreement with EPA, for the first time has demonstrated the critical importance of secondary transmission in disease outbreaks.

1999-2002
Demonstrated the metabolism of biochanin A and formononentin by human liver microsomes to the more estrogenic phytoestrogens genistein and daidzein. Isolated and characterized three hydroxylated formononetin derivatives, 6,7-diphyroxy-4'-methoxyisoflavone, 7-8-dihydroxy-4'-methoxyisoflavone, and 7,3'-dihydroxy-4'-methoxyisoflavone.

1999-2004
In response to a request from the Center for Food Safety and Applied Nutrition, determined the effect of topically applied glycolic and salicyclic acid on the carcinogenicity of simulated sunlight. The agents, which are alpha- and beta-hydroxy acids, are found in numerous cosmetics. The results indicated that glycolic acid did not effect the photocarcinogenesis of simulated solar light, and that salicylic acid was photoprotective.

Developed and validated a mechanistic approach to modeling the risk of liver tumors in mice exposed to fumonisin B1 in the diet. In addition to tumor data from the conventional NTP 2-year bioassay conducted at NCTR, the approach included data on tissue weights, cell proliferation, cell death, and sphingolipid metabolism in primary target organs. A two-stage, clonal-expansion cancer model produced predictions that indicated little or no risk at low doses of fumonisin B_1. The model's low-dose predictions provided scientific support and justification for the FDA's low-ppm guidance levels in corn products.

1999-present
Through an interagency agreement with the National Toxicology Program, National Institute for Environmental Health Sciences, established the National Toxicology Program Center for Phototoxicology. This facility allows rodent carcinogenicity studies to be conducted using terrestrial levels of light under controlled environmental conditions. The facility has been named one of four National Toxicology Centers for Research Excellence and is currently being used to assess the effect of light upon the carcinogenicity of number of chemicals of interest to the FDA.

1999-2005
Developed sensitive liquid chromatography/mass spectrometry methods for analysis of carcinogen-DNA adducts produced in animals by endogenous metabolism and exogenous chemicals.

Determined the metabolism and disposition of soy isoflavones in adult, neonatal, and fetal rats and mice.

2000
Laboratory building completed for ORA, Arkansas Regional Laboratories.

2000-present
Detected the presence of multiple antibiotic resistance markers in bacteria from different ecological backgrounds by the use of microarray methods.

Evaluated the composition and efficiency of probiotic products and their impact on the human intestinal microflora.

Investigated the role of intestinal microflora in conversion of plant hormones to either useful or ineffective metabolites.

Assessed the safety of drugs and other compounds involving understanding their effects on the gastrointestinal tract microbiota. We have been instrumental in the development of a decision tree for determining the limits on antimicrobial daily intake, which was adopted by the WHO and used in the FDA/CVM Guidance for Industry #52.

Evaluated the mechanisms of antimicrobial resistance of pathogens isolated from clinical, food, and environmental sources.

Developed intervention strategies (physical, chemical, or biological) to reduce the frequency, incidence and levels of multi-drug resistant microorganisms and other key pathogens in the U.S. food supply.

Provided the first evidence for the importance of genetic polymorphisms in chemotherapeutic drug metabolizing and detoxifying enzymes and therapeutic efficacy.

Developed an organ-specific carcinogenicity database for SAR analyses. Starting with over 25,000 records from the Carcinogenic Potency Database, a liver-cancer-specific database of 996 chemicals was built based on a hierarchical structure. SAR analyses of three sets of over 200 chemical descriptors each were conducted. A cross-validated concordance of 63% was achieved, but the sensitivity and specificity were directly influenced by the proportions of active and inactive chemicals in the database. The general dependency of sensitivity and specificity on imbalanced training data has been explored and documented, and analytical methods have been developed to reduce the influence of imbalanced data.

Established a rat model for studying the metabolic effects of, and biomarkers for, mitochrondrial dysfunction induced by the environmental toxicant, 3-nitropropionic acid.

2000-2001
Investigated sex differences in the inflammatory response to implanted biomaterials in rodents and found modest but significantly higher release of inflammatory mediators in the peri-implant area in females.

2000-2003
Established that riddelliine, a representative carcinogenic pyrrolozidine alkaloid, induced liver tumors in rats through the formation of exogenous DNA adducts.

Demonstrated the extrahepatic metabolism of biochanin A and formononetin. Notably, the cytochromes P450 1A1 and 1B1 are very active in metabolizing these substrates. This is important because cytochromes P450 1A1 and 1B1 are involved respectively in 2- and 4-hydroxylation of endogeneous estrogens that is thought to be important to the development of breast cancer.

2001-2004
Evaluated the antimicrobial resistance properties of probiotic products.

2001-2005
Designed and conducted studies to correlate UV treatments of newborn mice with acute photodamage and the subsequent development of cutaneous melanomas later in life using Tyr-Hras (+) Ink4a/Arf (+/-) transgenic mice.

Characterized male transgenic Tyr-Hras (+) Ink4a/Arf (-/-) mice as a model system for spontaneous transocular uveal melanoma.

Demonstrated significant modulating effects of soy-containing diets on adrenal and renal toxicity in rodents. These studies serve to underline the importance of carefully evaluating the diet used in preclinical toxicity studies.

2001-present
Optimization of tissue harvesting, freezing, sectioning, storage, and fixation protocols for high quality RNA retrieval from samples obtained after laser microdissection (LMD) technology, specifically, CK-7 IHC for stellate cells, GFAP IHC for bile duct cells, and GST-P IHC for preneoplastic foci in rat liver; Cox-1 and Cox-2 IHC for tubular cells in rat kidney.

Development of a protocol for genetic monitoring of mouse strains (C3H and C57BL) using micro satellite DNA analysis (typing by PCR).

Implemented procedures for assessing alterations in gene expression and function caused by drug exposure. Rodent models have been and will be utilized to help identify toxic responses to amphetamine and related compounds and other drugs of abuse (ecstasy), a variety of antiepileptic compounds (remacemide; phenytoin), metabolic inhibitors, (3-nitropropionic acid).

Designed and conducted studies to examine the carcinogenicity and tumor-promoting activities of the cosmetic ingredient and dietary supplement, Aloe Vera. The results of the short-term and sub-chronic studies on Aloe Vera indicated the colon as the major site of organ toxicity in the rat and mouse. The topical application of Aloe Vera to the skin of the SKH-1 hairless mouse enhanced the onset of skin lesions and the multiplicity of these lesions in long-term photo-carcinogenicity studies.

Developed a WINDOWS® based PBPK/PD software program. This user-friendly program uses a WINDOWS® interface for all input and output functions. Features include: four complete PBPK models in one program that can act independently or be metabolically linked; growth curves for rodents and humans that are utilized for organ/tissue volume and blood flow determinations; 20 doses for each input route of ingestion, iv bolus or infusion, dermal, ip, im, sc, and/or inhalation per simulation; metabolism via first order or Michaelis-Menten kinetics; elimination into urine, feces, and/or hair; weighted least squares regression algorithm for data fitting; and pharmacodynamic capabilities for both simple (e.g., adduct formation/repair) and complex (e.g., cholinesterase inhibition) interactions.

Developed statistical techniques for identifying genomic risk profiles. This included developing pre-processing methodology for normalization of microarray gene-expression data; and developing statistical theory for determining the number of genes that are differentially expressed with treatment, statistical methods for selecting optimal cutoffs for declaring significance, and statistical theory and methods for estimating associated false-discovery and false-non-discovery error rates. Work continues on the development of a statistical algorithm to compute adjusted p-values for correlated subsets of genes defined through a gene ontology, which can be used by FDA regulators in a systems approach to toxicity evaluation.

2002
Determined the metabolism and disposition of riddelliine, a carcinogen found in dietary supplements.

2002-2005
Developed sensitive liquid chromatography/mass spectrometry methods for the detection of benzo[a]pyrene DNA adducts and applied these techniques to individuals exposed to benzo[a]pyrene through cigarette smoke.

Proteomic-based microbiology projects resulted in the identification of novel proteins produced by mycobacterium involved in the catabolism of poly-aromatic hydrocarbons; and to identify virulence factors in antibiotic-resistant bacteria.

2002-present
Developed a comprehensive genomic database to identify and assess the biological threat of foodborne pathogens in the United States from imported food and feed products using conventional and state-of-the-art molecular tools, such as microarray bio-chip technology and real-time polymerase chain reaction (PCR).

Determined how endogenous host-derived factors, such as steroid hormones, bile acids, complex carbohydrates, and host immunity impact the microbiota and potential transient pathogens. Assessed the molecular genetics of pathogens isolated from clinical, food, aquaculture, and environmental sources.

Investigated the ecology, epidemiology, virulence, and molecular characteristics of foodborne pathogen populations and select agents for source tracking, delineating transmission pathways and better identifying targeted control measures in poultry, cattle, aquaculture, and clinical environments. We use molecular typing methods, such as pulsed-field gel electrophoresis, antibiogram patterns, multi-locus sequence typing, PCR-restriction fragment length polymorphism, ribosomal rRNA operon typing and ribotyping.

Developed capability of providing high-quality, web-based image archiving using DakoCytomation ScanScope and a large capacity (4 terabyte) server; a Virtual Microscopy/Pathology System (ScanScope), which allows digital storage of an entire microscope slide at diagnostic resolution. Capability of providing high-quality, web-based image archiving using DakoCytomation ScanScope and a large capacity (4 terabyte) server. This capability will allow consultation by off-site pathologists, the possibility of conducting the NTP

PWG's using the internet, and the ability to archive slide images on a local server for use by investigators in other divisions at the NCTR rather than transferring the slides themselves. The Virtual Microscopy/Pathology System (ScanScope) allows digital storage of an entire microscope slide at diagnostic resolution. The ScanScope system can be used for Pathology Diagnostic Consultation. The ScanScope system allows archiving in digital format of an entire study. Another prospective use for the ScanScope system would be in the area of Tissue Micro Arrays, which is an immerging technology that couples image analysis and immuno stained tissue sections. This system has the potential to be used in support of the phototoxicity studies where counting BrdU positive cells and other labeled cell types to access the relative toxicity of the dermal compounds. Scanning an entire skin tissue slide and the image of that slide is available to investigators to do cell counting, etc. This speeds up the process significantly and does not require the tissue slides to leave the custody of the pathology archives.
Developed esoteric testing in clinical pathology using Radioimmunoassay techniques counted on a 5 well gamma counter

Developed IHC methods to selectively distinguish spermatogonia and sertoli cells. Methods significantly improve the efficiency of collection accuracy of the data. Methods include dual staining with IHC, and PAS-H allows identification and quantitation of immunoreactive germ cell lineages in specific stages of the epithelium of the seminiferous tubule and sertoli cell nuclei negatively stain with PCNA antibody. All nuclei of germ cell lineages along basement membranes of seminiferous tubule stain positively by IHC, and an antibody specific for Protein Gene Product 9.5 selectively stains spermatogonia making them easily quantified by automated image analysis.

Established a Center for Functional Genomics to provide NCTR investigators and their collaborators with access to high-quality microarray technology for the investigation of biological mechanisms of action underlying the toxicity of products regulated by the FDA and related fundamental and applied research.

Established the Center for Proteomics whose initial work focused on improvements in throughput and sensitivity of mass spectrometry-based proteomic experiments as well as the development of bioinformatics to handle the large volume of data that are generated.
Established the Center for ToxicoInformatics to provide support to NCTR scientists, our sponsors and the regulatory community.

Investigated the carcinogenicity, genotoxicity (mutagenicity, micronuclei, and DNA incorporation), and metabolism of anti-retroviral drug combinations used to treated pregnant HIV-positive women to prevent the transmission of the virus to their babies.

Determined that irradiation of pigment yellow 74, a common pigment used in yellow tattoo inks, with simulated solar light results in photodecomposition to potentially toxic materials. These studies are currently being expanded to other commonly used tattoo inks.

ArrayTrack was developed to warehouse, visualize, analyze, and interpret microarray data. This integrated software solution is being extended to protein and metabolite data for Systems Biology questions.

2003-2005
Development of ProteinTrack, software that allows confidence criteria to be applied to proteomics results and comparisons made between different proteomics experiments.

Developed approaches, methodology, and standards for the determination of quantum dot fluorescence in tissue as a means to quantify the dermal penetration of quantum dots and the distribution of these nanoparticles to lymph nodes and other organs.

Determined the metabolism and disposition of endocrine disrupting chemicals in rats.

Determined the metabolism, disposition, and formation of DNA and hemoglobin adducts from acrylamide, a carcinogen formed in cooked food, and its reactive metabolite, glycidamide.

Determined the metabolism and disposition of HIV therapeutics in mice and monkeys.

Determined that a set of exogeneous DNA adducts can serve as biomarkers of tumorigenic pyrrolizidine alkaloids. Determined that metabolism of pyrrolizidine alkaloid N-oxides can also generate exogenous DNA adducts.

2003-present
Established a Center for Metabolomics to provide NCTR investigators with NMR-based metabolomic analysis and to provide internal expertise required to set standards in this new area. Procured and set up a tissue micro-array system to support ongoing and proposed experiments at the Center.

Apoptosis and proliferation counts were made by the use of virtual microscopy (Aperio ScanScope). Application of the nuclear algorithm provided by Aperio has been validated using experimental models of cycloheximide-induced apoptosis and partial hepatectomy-induced proliferation in the rat liver. Settings (parameters) of the algorithm were developed separately for apoptosis and proliferation counts. Use of virtual microscopy for the counts dramatically increased speed and accuracy of counting.

Optimization and application of apoptosis assays (TUNEL, ISOL and caspase-3 immunohistochemistry) for NCTR study of the modulation of DMBA-induced adrenal toxicity by soy-containing diet in female rats.

Developed, validated, and implemented automated data collection system for gross pathology (GPS) to allow for the collection and reporting of pathology data and tracking of specimens through the pathology division. System incorporates the gross pathology data, the in-house processing tasks (includes archiving), and in turn supports the Micropath system currently in use. System gives the ability to report automated gross pathology data to Principle Investigators immediately.

Established, in direct response to Agency needs, a rodent model for studying the consequences of lifetime exposure to low doses of acrylamide (a common contaminant of carbohydrate-based

foodstuffs cooked at high temperatures). These studies, supported by the National Toxicology Program, will have as a focus, the developmental neurotoxicity of acrylamide and will employ an extensive battery of simple and complex functional assessments of the nervous system. These assessments will include multiple behavioral tests as well as electrophysiological, neurochemical, and neuropathological analyses and occur throughout the lifetime of the animals.

Established, in collaboration with and support by NICHD and FDA colleagues, both *in vivo* and *in vitro* rodent and nonhuman primate models for assessing the consequences of developmental exposure to commonly used anesthetic agents (ketamine, benzodiazepines) on brain development. Not only will data generated from these studies directly affect the Agency's regulatory posture on these compounds, but they will also provide critical information on brain development by defining periods of sensitivity to drug exposure. Importantly, these studies will also provide the unique opportunity to directly assess the comparability of rodent and monkey models of brain development and perturbation. The National Toxicology Program is supporting the rodent part of the work.

Several novel concensus methods, including Decision Forest, were developed for diagnostic classifer and omics signature identification.

2004-2005
Proteomics is being used to identify serum biomarkers specific to liver cancer.
Involved in the design and conduct of studies to address outstanding questions regarding the risks of exposure of human infants to di(2-ethylhexyl)phthalate from medical devices.

Determined that photoirradiation of retinyl palmitate and its metabolites with UVA light generated reactive oxygen species (ROS) and resulted in lipid peroxidation.

2004-present
Developed RNA isolation protocols from samples obtained after laser microdissection (LMD) technology using hematoxylin or immunostained frozen tissue sections. Developed protocols for a sample preparation in RNase free conditions for laser microdissection of tissue.

Prepared and presented GLP training for the FDA in the BioResearch Monitoring Course for FDA auditors.

Developed statistical theory and methods for classification and risk prediction. This involved developing robust algorithms for classifying unknown/untested samples (tissues, chemicals) based on high-dimensional predictor data (genomic profiles, chemicals structures). In particular, the power of ensemble classifiers has been exploited to develop highly accurate classifiers that can be used with any type of input data, including mixtures of data from a variety of types. The algorithms have enormous potential to enable safe assignments of treatment therapies in personalized medicine and, consequently, sound regulatory decisions on newly proposed therapies to be made through risk/benefit decision analyses.

2005

Assisted the Center for Food Safety and Nutrition (CFSAN) in a Counter Bioterrorism study to assess the efficacy of heat treatments to reduce or eliminate the cytotoxicity of the biological warfare agent ricin added to infant formula preparations.

Showed that among diabetic patients, thiazolidinedione drugs reduce cancer risk by one-third, while insulin treatment increases cancer incidence nearly 70%**.**

A protocol for isolation of high-quality RNA after Laser Capture Microdissection (LMD) of hematoxylin-stained frozen sections was developed and optimized (in collaboration with Dr. Akihiro Naito).

The ArrayTrack training course was provided to the FDA reviewers/scientists.
Established an interagency agreement (IAG) with NIEHS to integrate ArrayTrack with the NIEHS CEBS system.

ArrayTrack was integrated into the VGDS Program with training for reviewers, analysis of submitted data, and formal discussions between CDER-NCTR and pharmaceutical sponsors.

2005-presesnt
The MAQC porject was initiated and completed with inclusion of all of the FDA Centers, the primary microarray providers, and the major RNA standards providers in order to provide quality control throughout the microarray processing and analysis process.

Established a three-year CRADA with SAS to integrate ArrayTrack with SAS SDS so that the statistical functionalities in SAS can be accessed through ArrayTrack.

Participated as one of the test sites for the MicroArray Quality Control (MAQC) project. The purpose of the MAQC project is to provide quality control tools to the broader microarray community in order to avoid procedural failures and to develop guidelines for microarray data analysis by providing the public with large reference datasets along with readily accessible reference RNA samples. The MAQC project involves six FDA Centers, major providers of microarray platforms and RNA samples, the EPA, NIST, academic laboratories, and other stakeholders. The MAQC project will help improve microarray technology and foster its proper applications in discovery, development, and review of FDA-regulated products.

Established, via an Interagency Agreement with the NIHCD, studies to examine the consequences of long-term exposure to methylphenidate on the learning performance of young (pre-adolescent) nonhuman primates (rhesus monkeys). Animals will be dosed, as are human children, and their ability to learn how to perform several cognitive function tasks will be monitored throughout a year-long period of treatment and for several months after treatment has been stopped.

Established a Systems Toxicology Division to facilitate integration of data from multiple technology platforms for application to questions associated with Critical Path Research, linking the five Centers of Excellence.

2006-present
A bioinformatics grant proposal was funded as a collaboration with UMDNJ-RW Johnson Medical School.