Biology & Cancer

Biology & Cancer – Addressing tobacco toxicology, cancer susceptibility, and biologic gender differences related to tobacco and cancer

Over 60 studies address laboratory and clinical efforts to understand the biology and toxicology of nicotine as they pertain to women. Smoking continues to be examined in clinical studies as a modifier of risk for breast and other cancers in women. Links between smoking and other diseases are also being investigated. The biological effects of smoking during pregnancy, both for the mother and the child, are being investigated in a number of studies.

Smoking and Cancer

While it has been long established that smoking is a cause of cancer, currently funded research seeks to extend our knowledge. The relationship between smoking and the rate of lung metastases is being investigated in mice as a possible explanation for why breast cancer may behave more aggressively in smokers. Other investigators are determining whether a subset of women with atypical metabolism of smoke products is at higher risk for breast cancer. Male and female mice exposed to tobacco smoke are being examined for cellular changes in focal lung regions to explain a greater susceptibility of women to lung cancer. Several clinical studies on causes of breast, colorectal, ovarian, anogenital, cervical, and lung cancer take smoking into account as a potential modifying factor in risk of developing these cancers.

Smoking Effects Other Than Cancer

In addition to its carcinogenic effects, smoking affects a number of systems. Researchers are studying the effects of tobacco smoke on arterial disease in a postmenopausal mouse model. Another group is investigating a link between tobacco smoke and progressive kidney disease, and the role of estrogen in this process. The link between smoking and elevated homocysteine levels is being investigated as a possible explanation for increased rates of cardiovascular disease in smokers. Smoking may also exacerbate osteoporosis and act as a toxin to the reproductive endocrine environment. To investigate the latter, investigators are examining the effect of smoking on ovarian steroid secretion and progesterone clearance. Another study proposes to examine the effect of smoking on menstrual function by tracking follicle-stimulating hormone on a daily basis. Research is being conducted on the effect of mainstream and sidestream smoke on the oviduct and corpus luteum, using a hamster model. Smoking may also affect the brain. Researchers at the University of California recently studied the association between smoking, estrogen, and cognitive function. One study used a rodent model of adolescent nicotine administration to determine if smoking affects the set point for activity of the neurotransmitter 5-hydroxytryptamine. A second study used proton magnetic resonance spectroscopy to determine that nicotine affects the activity of glutamate and myoinositol. Investigators at the University of Pittsburgh are examining how the effects of smoking and acute stress on the immune and sympathetic nervous systems converge in women. Other investigators are testing whether topical testosterone and strength training for women with chronic obstructive pulmonary disease can improve performance of the muscles involved in breathing. Another study is examining the relationship between smoking and the frequency and severity of premenstrual symptoms.

Gender Differences in Smoking/Response to Nicotine

Investigators are using a mouse model to determine whether there are gender differences in the cellular and genetic changes that occur in response to tobacco smoke. Other investigators are testing whether women crave the taste of smoke more than men; they also will determine the effects of withdrawal across the menstrual cycle. Another group is using a rat model to determine whether gonadal hormones act on dopaminergic systems to sensitize females to nicotine.

Effects of Nicotine/Smoking During Pregnancy on Fetus/Child

A number of studies are examining the effects of smoking during pregnancy on development in human and animal models. One major area of focus is the effects of smoking on brain development. One study recently examined the effects of nicotine on development of the auditory cortex in a rat model. Investigators are researching the effects of nicotine and smoking on neural cell apoptosis (programmed cell death), activity of the neurotransmitter 5-hydroxytryptamine, catecholamine response to hypoxia during delivery, nicotine receptors that regulate dopamine, and inhibition of growth factor regulation and subsequent growth deficits. Other groups are also determining the effect of nicotine on postnatal vulnerability to spontaneous abortion, sudden infant death syndrome, attention deficits, impairment of regulatory processes, and other disorders. A comparison of the effects of using snuff and smoking on pregnancy outcomes is being examined in a retrospective study. Several studies are examining the long-term effects of prenatal smoking on the development of behavioral problems in children, including delinquent behavior and aggression. A nicotine vaccine given to the mother is being investigated in rats for its ability to protect the fetus from harmful effects of the drug. A second major area of focus deals with the effects of nicotine on the developing lung. Investigators are examining the effects of nicotine on fetal lung growth, gene expression, and nicotine receptor levels. Other researchers are testing the protective effect of retinoic acid on lung development, as well as the effects of passive smoke exposure on lung growth and development. A third major area of focus is the mutagenic and carcinogenic properties of tobacco smoke and nicotine on the fetus and child. Investigators are using accelerator mass spectrometry to measure DNA and protein adduct levels in offspring of smoking mothers. Transplacental pancreatic carcinogenesis by NNK, a substance found in tobacco smoke, will be measured. Investigators are also examining the interaction of tobacco smoke and naturally occurring genetic variations, and measuring DNA adducts in tissues of suckling infants after administering a carcinogen to mothers. Researchers at the University of California are examining the vulnerability of the placenta to toxic effects of maternal tobacco use.

Title: Mechanisms in Perinatal Carcinogenesis
Principal Investigator: Anderson, Lucy M.
Institution: National Cancer Institute Frederick Cancer Research Center, Frederick, MD
Funding Agency: National Cancer Institute
Project ID: BC005352
Project Funding Period: Not available

Abstract: Perinatal exposures may lead to increased risk of childhood cancers, as well as those later in life. Preconceptional parental, transplacental, and/or neonatal exposures may be involved. Studies with animal models are utilized to increase understanding of underlying cellular and molecular mechanisms. Transmammary neonatal exposures have received relatively little attention. We have studied exposure by this route to the carcinogenic nitrosamine, N-nitrosodimethylamine, which is present in tobacco smoke and other environmental sources. After administration to lactating rat mothers, N-nitrosodimethylamine caused formation in

tissues of suckling infants of a DNA adduct known to be associated with tumor initiation. Furthermore, if the mother received ethanol at the same time, there was a 10-fold increase in these adducts in some tissues. These results indicate that transmammary exposures should receive further study. Another understudied exposure issue is that of transgenerational carcinogenesis. We are examining the effects of paternal exposure to chromium (III), a chemical widely encountered occupationally. Exposure of male mice to this chemical before mating leads to increased incidence of several types of neoplasms in the offspring. Preliminary results indicate that this is associated with changes in serum hormones in the offspring, and altered expression of a number of hepatic genes as studied by microarray, including insulin-like growth factor binding protein 1.

Title: Hormone Replacement Therapy, Smoking & Lipoprotein Oxidation
Principal Investigator: Banka, Carole
Institution: La Jolla Institute for Molecular Medicine, La Jolla, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 7RT-0101
Project Funding Period: 1 July 1998 – 30 June 2001

Abstract: When women reach menopause, their risk for heart disease increases, in part, because of the aging process and, in part, because their ovaries no longer make estrogen and progesterone. Estrogen is known to reduce cardiovascular risk significantly. Cigarette smoking constitutes a third significant risk factor in postmenopausal women and environmental tobacco smoke (ETS) also contributes to cardiovascular risk in "passive" smokers. Therefore, it appears that the lack of ovarian hormones associated with menopause and exposure to cigarette smoke (passive or active) constitute the two most important risk factors for heart disease in otherwise healthy women. Hormone replacement therapy (HRT), the replacement of estrogen (or estrogen and progesterone) through medication, reduces the risk of heart disease by as much as 50% in postmenopausal women. Yet physicians are hesitant to prescribe HRT for women smokers, due to the historical (albeit rare) occurrence of blood clotting (and the resulting risk of blood vessel occlusion and/or stroke) in women who smoke and take contraceptive pills that contain estrogen. However, the risk of clotting associated with HRT is minimal and increases only slightly in HRT-treated women who smoke. Although the ideal approach to management of heart disease risk in postmenopausal women would include smoking cessation, for those women who chose to continue smoking, or who live in a situation associated with exposure to environmental tobacco smoke, HRT may be an especially important intervention. It is my goal to develop a small animal model for the first rigorous studies of the interactions between environmental tobacco smoke, postmenopausal status and hormone replacement therapy as they relate to heart disease. To this end, I have designed this proposal to accomplish the following specific aims: Aim #1 To test the hypothesis that exposure to environmental tobacco smoke (ETS) will accelerate artery disease in normal mice and to a greater extent, in mice whose ovaries have been removed (postmenopausal mice). Aim #2: To test the hypothesis that estrogen, and estrogen + progesterone, will partially reverse the acceleration in artery plaque formation resulting from removal of the ovaries and from ETS exposure. Aim #3: To test the hypothesis that factors involved in ETS-induced acceleration of artery disease include oxidant stress in addition to increases in cholesterol levels, and that cholesterol levels and oxidant stress are reduced as a result of HRT.

The successful completion of these aims will yield valuable information concerning 1) the extent and mechanisms of ETS-mediated acceleration of arterial disease; 2) the additive effects of ETS and postmenopausal status on risk of heart disease; 3) the mechanisms underlying protective effects of estrogens; and 4) the interactions of ETS and hormone replacement therapy.

Title: Smoking, Stress, and Immune Function
Principal Investigator: Baum, Andrew
Institution: University of Pittsburgh at Pittsburgh, Pittsburgh, PA
Funding Agency: National Institute on Drug Abuse
Project ID: DA010887
Project Funding Period: 20 February 1997 – 31 January 2002

Abstract: There is considerable evidence that behavioral factors such as smoking, drug-taking and psychological stress have an important impact on the risk of cardiovascular and immune-related diseases, including infectious and neoplastic disorders. Recent findings also suggest that these factors can promote viral infections and accelerate the development of some AIDS-related infections in HIV seropositive individuals. Given the potential health impact of these risk factors in both normal and at-risk (e.g., HIV positive) populations, there is need for more basic research on the mechanisms mediating their cardiovascular and immune actions. Moreover, these factors rarely occur individually outside of the laboratory, and a more realistic laboratory model should include their convergent, in addition to their isolated effects. Finally, considerably more research has been devoted to the effects of these variables in men and more information is needed on their consequences in women. Our research team has had considerable experience in studying the endocrine, cardiovascular and immunological effects of smoking and stress in men and women and the endocrine and immunological effects of nicotine in laboratory animals. On the basis of our research, and the findings of others, we propose that smoking and stress are convergent risk factors for cardiovascular and immune-related disease and that their convergent effects may be mediated by acute activation of the sympathetic nervous system. We now propose to extend our research to study the convergent effects in women of two of these factors, psychological stress and smoking. Specifically, we will determine: 1) how the effects of smoking and acute stress on the immune and sympathetic nervous systems converge in women and 2) how the convergent effects of smoking and stress in women are altered by smoking cessation. The first goal will be achieved by comparing the effects of acute laboratory stress on smokers, with and without concurrent smoking, to the effects of stress in non-smokers. The second will be approached by using an already planned and funded smoking treatment program to assess baseline and stress-induced changes in the function of the SNS and immune systems which accompany cessation of smoking in women.

Title: Metabolism and Clearance of Nicotine in Pregnant Women
Principal Investigator: Benowitz, Neal L.
Institution: University of California, San Francisco, San Francisco, CA
Funding Agency: National Center for Research Resources
Project ID: RR000083-380325
Project Funding Period: Not available

Abstract: The specific aims of this study are to determine: (1) metabolic renal clearance of nicotine and cotinine; (2) the plasma half-life of nicotine and cotinine; (3) fractional conversion of nicotine to cotinine; and (4) pattern of nicotine metabolism in pregnant women. Investigators will also determine alterations in tobacco use (nicotine intake) that may occur during pregnancy.

Title: Mentored Investigator Award in Women's Health
Principal Investigator: Boardman, Lori A.
Institution: Women and Infants Hospital-Rhode Island, Providence, RI
Funding Agency: National Institute of Child Health and Human Development
Project ID: HD001307
Project Funding Period: 25 September 2001 – 31 August 2006

Abstract: The purpose of this award is to provide support for Dr. Lori Boardman to pursue formal training in the fields of biostatistics, epidemiology and public health, thereby attaining the necessary theoretical and methodological background to further a career in patient- oriented research. The final three years of the award will be devoted to the design, implementation, analysis of data and preparation of the results of a randomized controlled trial of two smoking cessation interventions in a cohort of women referred for the evaluation of abnormal Papanicolaou smears. The primary aims of this study are to evaluate smoking cessation rates between the two groups and to confirm self-reports of cessation through measurement of cervical mucus cotinine. The secondary aims are to determine the regression rate of cervical neoplasia in women who quit smoking compared to those who continue and to assess the independent and combined contribution of human papillomavirus and smoking on the natural history of atypical or low-grade cervical neoplasia (includes cytology and/or histology). This trial will be conducted with the guidance of a multidisciplinary and experienced team including experienced women's health and behavioral health researchers, epidemiologists, an oncologist, and a statistician. Immediate Career Objectives: Pursue formal training in research design and analysis by obtaining a master's degree in public health; Improve abilities to design, perform, analyze and communicate research findings through the preparation of a master's thesis and formal presentations of ongoing research stemming from clinical work in cervical neoplasia; Implement and complete a randomized trial of two smoking cessation interventions in women with cervical neoplasia. Lone-Term Career Objectives: Become an independent and productive investigator in the field of women's health care; Secure independent grant funding for patient-oriented research; Become a leader in academic medicine and mentor more junior investigators interested in women's health.

Title: DNA Damage in Human Endometrium by Tamoxifen Treatment
Principal Investigator: Bodell, William J.
Institution: University of California San Francisco, San Francisco, CA
Funding Agency: National Cancer Institute
Project ID: CA075402
Project Funding Period: 4 September 1998 – 31 August 2002

Abstract: Tamoxifen (TMX) is an antiestrogenic compound used in the treatment of breast and other cancers. Women treated with TMX have been reported to show up to a 6-fold increased risk for the development of uterine cancer. Studies from the applicant's laboratory suggest that TMX usage may result in the formation of DNA damage in human endometrium. In these studies the applicant proposes to: (1) collect endometrial biopsy samples from a group of women with varying TMX usage. Patient medical records will be used to asses TMX usage and a questionnaire will be used to analyze other variables including age, menopausal status, and tobacco history. (2) Measure levels of TMX metabolites and uterine peroxidase activity in the endometrial samples. These measurements will be treated as biomarkers of internal TMX dose and individual capacity to activate TMX metabolites to form DNA damage. (3) Measure levels of DNA adducts and 8-hydroxydeoxy-7,8- dihydroguanosine in the endometrial samples Attempts will be made to verify that the adducts detected in the endometrial samples are derived from TMX by direct analysis with liquid chromatography-mass spectrometry. (4) Identify the structure of the DNA adduct formed by 4-OH-TMX. (5) Develop a model to test for associations

between TMX usage and the formation of DNA adducts and 8-hydroxydeoxy-7,8-dihydroguanosine in endometria samples. The applicant will seek to determine if any association is modified by either levels of TMX metabolites in endometrial samples, activity of uterine peroxidase, or tobacco smoking history. Statistical power has been considered regarding the hypothesis that administration of TMX results in the formation of DNA damage in human endometrium.

Title: Neurodevelopmental Basis(es) of Nicotine Sensitization
Principal Investigator: Booze, Rosemarie M.
Institution: University of South Carolina at Columbia, Columbia, SC
Funding Agency: National Institute on Drug Abuse
Project ID: DA013712
Project Funding Period: 28 September 2002 – 30 June 2007

Abstract: Gender differences in response to psychostimulants have been reported both in animals and humans; however, the biological mechanisms which underlie these gender differences to psychostimulants remain for the most part, unexplained. The common observation is that females are more sensitive to psychostimulants, such as nicotine. Our hypothesis is: Gonadal hormones in adulthood and development act on dopaminergic systems, providing the underlying basis for the gender differences in behavioral sensitization produced by repeated IV nicotine administration. First, we will determine whether pharmacokinetic differences between the sexes result in higher levels of nicotine in the female brain. We have successfully developed a technically simple, economical and practical non-tethered technique for repeatedly administering IV nicotine to freely moving, group-housed rats. Detailed pharmacokinetic analysis has demonstrated rapidly peaking nicotine levels following IV dosing in rats, which is similar to that observed in humans, as opposed to SC or PO dosing. Using this clinically relevant IV rodent dosing model, we will determine whether pharmacokinetic factors contribute to the increased sensitivity of female animals to the effects of nicotine. Second, we will determine whether gonadal hormones regulate the expression of gender differences in response to nicotine in adulthood. We will test the ability of gonadal hormones to modulate dopamine receptor responsiveness to chronic nicotine administration. Third, we will determine whether the brain organizational (neurodevelopmental) effect of the perinatal hormonal milieu mediates the gender differences in nicotine responsiveness. We have pharmacologically characterized a recently discovered unique dopamine receptor subtype (D3) which is localized to the striatum/nucleus accumbens region of the brain. We hypothesize that alterations in dopaminergic systems underlie the gender differences produced by repeated IV nicotine administration. Our long-term goal is to determine the role of the dopamine neurochemical system in gender differences following repeated IV nicotine administration. The ultimate goal of this research is to develop pharmacological interventions to assist in correcting the behavioral problems associated with chronic tobacco use in humans, and specifically to provide potential insight into effective gender-specific treatment strategies for smoking cessation.

Title: Fetal Alcohol and Nicotine Induced Growth Retardation
Principal Investigator: Breese, Charles R.
Institution: Auburn University at Auburn, Auburn, AL
Funding Agency: National Institute on Alcohol Abuse and Alcoholism
Project ID: AA011164
Project Funding Period: 21 September 1998 – 31 August 2002

Abstract: Fetal alcohol syndrome is a constellation of birth defects caused by maternal alcohol use during pregnancy, and is characterized by intrauterine and postnatal growth deficits, and

CNS dysfunctions in the offspring. Tobacco use during pregnancy is also an established cause of fetal growth deficiency, although the toxicological effects of prenatal nicotine exposure on the CNS are not clear. Since tobacco use is highly correlated in women that abuse alcohol during pregnancy, exposure to the combination of these substances may exacerbate the deficiencies associated with alcohol or tobacco use alone. While intrauterine and postnatal growth deficiencies are the most common symptoms of fetal alcohol or tobacco exposure, the cause of these deficiencies unknown. Studies have shown a consistent long-term reduction of insulin-like growth factor-1 (IGF-1), a major mediator of developmental growth, in prenatally ethanol-exposed offspring. The goal of this application is to investigate the actions of in utero ethanol, nicotine, and ethanol/nicotine co-exposure, on the regulation of the IGF and somatotropin gene families, and assess the relationship of changes in tissue and brain IGF and GH regulation, to that of the growth and CNS deficits observed in these offspring. The hypothesis is that fetal exposure to ethanol and nicotine inhibits fetal and neonatal IGF-1 gene expression, thereby reducing tissue availability to IGF-1, and causing or exacerbating the observed growth deficits observed in these offspring. The proposed studies to test this hypothesis include: 1) Examining the effect of fetal ethanol, nicotine and alcohol/nicotine co-exposure, on plasma and somatic tissue specific IGF and GH peptide and gene regulation; 2) Assessing the effect of fetal ethanol and nicotine exposure and co-exposure on changes on CNS neurotrophic expression, with particular emphasis on the IGF and neurotrophic gene families; 3) Examining the specific actions of ethanol and nicotine exposure on growth factor induced cellular function and second messenger systems, in organ culture systems of affected tissues; and 4) Assessing changes in gene expression by differential display PCR, to identify additional candidate genes in these disorders. These studies will provide valuable data which correlate with the endocrine and neuropathological changes seen in fetal alcohol syndrome and smoking in human populations.

Title: Anabolic Therapies for Muscle Dysfunction in Women with COPD
Principal Investigator: Casaburi, Richard
Institution: Harbor-UCLA Research & Education Institute, Torrence, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 10RT-0046
Project Funding Period: 1 July 2001 – 30 June 2004

Abstract: This project aims to improve the effectiveness of medical treatment for women with lung disease produced by cigarette smoking. Chronic obstructive pulmonary disease (COPD, also called emphysema or bronchitis) is a disorder affecting approximately 14 million people in the United States. It is a disabling disorder and inability to exercise is usually the foremost problem. It is becoming clear that these patients suffer not only from poorly functioning lungs but also because the muscles of ambulation perform poorly. We have recently shown that muscle function can be improved in men with COPD both by strength training and by giving supplemental testosterone, a drug known to build muscles. Until now, most research into methods to improve muscle function has involved only men. However, the number of women suffering from COPD is accelerating and is nearly equal to that of men. The body chemicals that stimulate muscle growth operate differently in women than they do in men. We will focus on two specific strategies intended to improve the muscle's ability to tolerate exercise. First, there is a growing realization that testosterone is not only a “male hormone.” Though levels in women are much lower than in men, studies in healthy women suggest that testosterone supplementation increases muscle size. We have found that women with COPD have low levels of this chemical. However, the amount of testosterone that best balances the benefits of testosterone without creating troubling side effects has yet to be determined. We will determine whether administering testosterone to women with COPD will increase muscle mass and exercise tolerance. Second, we have found that women with COPD usually have a low level of

activity; we will determine whether a strength conditioning program consisting of a varied group of exercises improves exercise tolerance. We will assign by chance 72 women with COPD to one of six groups. Four of the groups will receive no training, but will apply a gel to their skin daily that will contain no testosterone or one of three doses of testosterone. Two of the groups will receive strength training (for one hour a day three times per week) plus either a gel with no testosterone or with the highest of the three doses of testosterone. A number of state-of-the art measurements will be made before and after the 10 week study period including 1) precise measurement of the muscle and fat mass in the body, 2) muscle strength by weight lifting and by measuring the electrical activity of the muscle, 3) exercise capacity measured on a stationary bicycle, 4) strength of the breathing muscles and 5) the overall quality of life, assessed by well-designed questionnaires. In this study, a number of safeguards will be in place to assure the participants' safety. Programs are already in place to help rehabilitate patients with COPD. This study should be directly applicable to these programs and will help to decrease the suffering of patients with this smoking-related disease.

Title: Nicotine and Brain Development
Principal Investigator: Chen, Wei-Jung A.
Institution: Texas A&M University Health Science Center, College Station, TX
Funding Agency: National Institute of Neurological Disorders and Stroke
Project ID: NS039899
Project Funding Period: 1 April 2000 – 31 March 2005

Abstract: The overall purpose of this proposal is to evaluate developmental nicotine-induced neurotoxicity. Despite the Surgeon General's warning concerning the harmful effects of smoking on the developing fetus, there is still a disturbing number of pregnant women who smoke during pregnancy. This proposal will utilize a rat model system to examine how and to what extent nicotine affects the developing brain. The proposal will test several hypotheses that are categorized into three Specific Aims. Specific Aim number 1 will test two hypotheses: 1) that nicotine exposure during all three trimesters equivalent will result in region-specific reductions in neuronal numbers in two important brain regions (hippocampus and cerebellum) in neonates and young adults; and 2) that the long-term brain deficits resulting from developmental nicotine exposure will be manifested through and correlated with specific behavioral impairments, spatial learning and parallel bars tasks, respectively. The exposure regimen used in Specific Aim number 1 is especially clinically relevant, since most pregnant women who smoke do so throughout pregnancy. Specific Aim number 2 will test two hypotheses: 1) that nicotine exposure during the third trimester equivalent (the brain growth spurt period) will lead to more severe neuronal loss than exposure restricted to first or first and second trimesters equivalent, and 2) that the cessation of nicotine exposure gestation will be beneficial to the developing brain. Specific Aim number 2 is important in addressing the questions regarding temporal vulnerability and the potential interaction between brain-regional specificity and temporal factors in mediating differential effects on nicotine-induced neuronal loss. Specific Aim number 3 will begin to address the question of mechanisms underlying nicotine-induced neuronal loss by testing the hypothesis that the application of specific neurotrophic factors (brain-derived neurotrophic factor [BDNF] and glial-derived neurotrophic factor [GDNF]) will attenuate nicotine-induced neuronal loss in an organotypic explant culture system. Specific Aim number 3 is the fist step to identify the involvement of specific neurotrophic factors as one of the underlying mechanisms for developmental nicotine-induced neuronal loss. The proposal will incorporate innovative in vivo and in vitro approaches to evaluate nicotine's toxicity during brain development, and many of the experimental techniques (artificial-rearing for third trimester equivalent exposure, 3-D stereological cell counting, organotypic explant culture system) proposed to be implemented in this proposal are novel to developmental nicotine research. The proposed studies will contribute to and broaden our knowledge of the harmful consequences from maternal smoking during

pregnancy, provide a better understanding of the potential risk that may influence the severity of nicotine-induced brain deficits during different stages of development, and lead to a focus on mechanistic issues regarding developmental nicotine-induced neurotoxicity.

Title: Effects of Nicotine on Brain Development Assessed by 1 H MRS
Principal Investigator: Cloak, Christine
Institution: Cedars Sinai Medical Center, Los Angeles, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 8DT-0170
Project Funding Period: 1 January 2000 – 31 December 2001

Abstract: Maternal cigarette smoking produces profound health effects including premature labor, low birth weight, stillbirth, and neonatal death. Postnatal growth and behavior also are affected. Despite all the public health warnings many pregnant women still smoke or are placed on nicotine replacement therapies such as the patch. Nicotine is well known as an important component of cigarette smoke and has been implicated in many of the adverse effects of smoking, on fetal development. Although data clearly show that prenatal exposure to nicotine is bad, considerably less is known about the effects of nicotine on the brain during other developmental periods, particularly during puberty. The brain is still maturing rapidly during puberty and, most likely, it remains vulnerable to the detrimental effects of nicotine during this period as well. We extended our studies to determine if the "window" of vulnerability that exists during very early development extends into adolescence. We used a relatively new imaging procedure called proton magnetic resonance spectroscopy (1 H MRS), which can be performed safely on humans. One of the brain metabolites measured by 1 H MRS is the compound n-acetyl-aspartate (NAA). In animals with brain damage or humans with neurodegenerative diseases, NAA concentrations are reduced, in a regionally specific manner. The concentration of this brain metabolite reflects the health and viability of neurons. We hypothesized that developmental nicotine exposure would result in a decrease in NAA concentrations. Our data indicated that NAA concentrations were not reduced in adult offspring exposed to nicotine during development, however other brain metabolites were affected. The primary focus of the study (long-term effects of developmental nicotine exposure) has been completed and written up as the Ph.D. dissertation for Christine Cloak. We are in the process of converting the dissertation into journal, articles for publication. Tissue has been collected but not yet analyzed for the progressive developmental effects of nicotine exposure (early developmental time points). Although our hypotheses concerning the long-term effects of nicotine during development on NAA concentrations in the brain were not supported, other measurable metabolites were effected such as glutamate and myo-inositol. Future studies will focus more on these metabolites.

Title: Dietary and Hormonal Determinants of Cancer in Women
Principal Investigator: Colditz, Graham
Institution: Brigham and Women's Hospital, Boston, MA
Funding Agency: National Cancer Institute
Project ID: CA87969
Project Funding Period: 12 September 2000 – 30 November 2004

Abstract: The overall long-term objective of this Program Project is to identify novel dietary and hormonal determinants of breast colorectal and ovarian cancer risk in women, with the ultimate aim to find means for prevention and improved survival. The combination of questionnaire derived data with biomarkers, coupled with the long-follow-up, affords the opportunity to further understanding of the time course and mechanisms of cancer development. To achieve these objectives, we will relate a) prospectively collected data on diet, post-menopausal hormone use, smoking, and other behaviors; b) nutrient and hormone levels in prospectively collected blood; and c) genotyping information form archived DNA and tissue

blocks; to incidence of breast colorectal, and ovarian cancer. This Program Project is based on the Nurses' Health Study cohort comprising 1212,700 women who were 30 to 55 years of age when enrolled in 1976. The Program Project serves as the central resource for the many related grants addressing incidence of cancer and other major chronic diseases that arise in this cohort of women. Project 1. Diet, exogenous hormones and breast cancer risk. Project 2. Diet, hormones and brisk of colorectal cancer. Project 3. Hormones, diet and risk of ovarian cancer. Project 4: Statistical innovations in risk modeling.

Title: Anogenital Cancer--Epidemiology/Biochemistry/Immunology
Principal Investigator: Daling, Janet R.
Institution: Fred Hutchinson Cancer Research Center, Seattle, WA
Funding Agency: National Cancer Institute
Project ID: CA042792-150004
Project Funding Period: Not available

Abstract: We propose to study risk factors for multiple primary anogenital cancers and to continue our case-control studiers of cervical and vulvar cancer. Both studies have as their goal the elucidation of factors, beyond HPV, that contribute to the etiology of anogenital cancer. The new, major focus of this proposal will be risk factors for the development of multiple primary anogenital tumors. We will match each of these women to a women with one anogenital tumor who does not go on to develop a second tumor. We will interview multiple primary anogenital cancer cases and matched single primary controls about characteristics that may be related to their risk of a second primary tumor, and collect archival tumor tissue to test for HPV DNA types and non- prototype variants. Blood will be collected for serologic and genetic testing. We plan to determine whether the risk of multiple primary anogenital cancers is related to: 1) cigarette smoking, particularly continued smoking following the initial primary; 2) HLA class II alleles; 3) family history of anogenital cancers; 4) HPV type and non-prototype variant in the initial primary cancer. The data from this study may contribute to the design of clinical monitoring for anogenital cancer patients at high risk of second primary cancer, and may provide targets for behavior modification that could reduce the incidence of multiple anogenital tumors. In continuing our case-control study, we will example HPV co-factors in relation to risk cervical and vulvar carcinoma. The study will be conducted in three counties of western Washington. All women engaged 18-74 who are diagnosed from January 2000 through December 2004 with cervical or vulvar cancer will be identified through the population- based Cancer Surveillance System. Cases and population-based controls will be interviewed regarding history of sexually transmitted diseases, smoking status, family history of anogenital and other cancers, as well as known risk factors for each tumor. Tissue specimens will be obtained from all cases and will be assayed for HPV DNA. Blood will be collected and tested for antibodies to HPV and for HLA alleles. The data will provide sufficient power for testing important interactions among HLA alleles, and between HLA alleles and lifestyle risk factors.

Title: Tobacco: Prenatal Effects and Adolescent Use
Principal Investigator: Day, Nancy L.
Institution: University of Pittsburgh at Pittsburgh, Pittsburgh, PA
Funding Agency: National Institute of Child Health and Human Development
Project ID: HD036890
Project Funding Period: 3 September 1998 – 31 May 2003

Abstract: The Maternal Health Practices and Child Development Project is a prospective study of the effects of prenatal tobacco exposure on the offspring of 755 women. We have identified significant effects of prenatal tobacco exposure on the offsprings' development of the central

nervous system and on delinquent behavior and peer problems. At 10 years of age, the children have begun to experiment with tobacco and other substances. These children were more depressed and anxious, they had more attention problems, aggression, and delinquency. We will assess the long-term effects of mental and physical development, temperament, psychological status, activity levels, academic performance, behavior problems, the environment, and prenatal exposure on the adolescents' substance use at age 14 and 16 and on the development of tobacco use between the ages of 14 and 16. No prior study has been able to explore the predictors of adolescent tobacco use across time, from birth to adolescence. The cohort is a general population sample of low income women selected from a prenatal clinic. Half the women are Caucasian, half are African-American. We have assessed these mothers and their children at prenatal months 4 and 7, delivery, 8 and 18 months, 3, 6, and 10 years. We have an exceptional follow-up rate of 91% at 10 years.

Title: Chronic Progressive Kidney Disease and Exposure to Cigarette Smoke in the Post-Menopausal State
Principal Investigator: Elliot, Sharon
Institution: Vascular Biology Institute, University of Miami School of Medicine, Miami, FL
Funding Agency: Florida Tobacco Control Program
Project ID: Not available
Project Funding Period: Not available

Abstract: There is clear evidence that cigarette smoking is associated with an increased risk for progressive kidney disease in both men and women. Unfortunately, there have been few studies of the mechanisms involved in this important problem. Interestingly, the renal diseases involved have been found to be very diverse and include primary vascular diseases such as hypertension, metabolic diseases affecting glomeruli such as diabetic nephropathy, and genetic diseases affecting tubules such as polycentric kidney disease. In addition, data from the United States Renal Data System reveals that certain risk factors aggravate renal injury, and that estrogen deficiency compounds the effect of these risk factors on renal disease. Since the number of aging women in the population is increasing, and the number of women who are smokers is also increasing, the relationship between estrogen deficiency and smoking can now be considered to be a significant health care issue. The specific aims are as follows:

- Aim 1. Determine whether exposure to cigarette smoke induces glomerular changes in young B6 mice that are characteristic of the aging kidney and whether estrogen deficiency contributes to induction or aggravation of these vascular pole lesions.

- Aim 2. Determine whether estrogen replacement ameliorates the vascular pole lesions in aging B6 mice.

- Aim 3. Determine whether mesangial cells isolated from the mice in aims #1 and #2 demonstrate stable phenotypic changes in vitro and whether they are associated with changes in estrogen responses, and whether estrogen replacement prevents these changes.

Title: Psychological Influences on Immune Responses to HPV
Principal Investigator: Fang, Carolyn
Institution: Fox Chase Cancer Center, Philadelphia, PA
Funding Agency: National Cancer Institute
Project ID: CA88307
Project Funding Period: 1 August 2000 – 31 July 2002

Abstract: The role of certain types of human papillomavirus (HPV) in the etiology of cervical cancer is well-established. However, the influence of psychosocial, behavioral and immunologic factors on cancer risk and development needs further exploration. The proposed project aims to elucidate the potential links between psychological (e.g., distress, coping processes) and behavioral (e.g., cigarette smoking) risk factors and novel immunologic measures (e.g., T-cell proliferative responses to HPV proteins) in women with mild dysplastic lesions of the cervix due to infection with highly oncogenic subtypes of HPV. Specifically, the proposed project is designed to identify potential behavioral and immunologic correlates of psychological distress and coping, with a particular emphasis on the effects of avoidant coping strategies on cancer risk and development. Sixty-two women referred for a follow-up colposcopy will complete baseline psychosocial assessments and provide a blood sample (for immune assays) prior to their colposcopy. In addition, HPV typing of cervicovaginal cells will be conducted at baseline. Follow-up assessments will be conducted at 6-months and 12-months post-baseline. Psychosocial assessments include measures of psychological distress, cancer-specific intrusive and avoidant ideation, and a variety of coping strategies. Relevant immune measures include numbers and percentages of circulating lymphocytes, as well as T-cell proliferative responses to synthetic peptides derived from HPV 16, a specific marker of immunocompetence and one that has been shown to be associated with viral clearance and cervical disease regression. In addition, medical outcome (regression, persistence, or progression of cervical lesions), demographic variables, and behavioral risk factors (e.g., smoking) will be assessed. The identification of potential interrelations among psychosocial, behavioral, and immunologic variables has important implications for cancer prevention and control, as this information can be used to guide the development of psychological and behavioral interventions aimed at reducing distress and avoidance, which may lead to improved behavioral, immunologic, and health outcomes.

Title: Effect of Maternal Smoking on Human Placental Development
Principal Investigator: Fisher, Susan
Institution: University of California, San Francisco, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 9RT-0112
Project Funding Period: 1 July 2000 – 30 June 2003

Abstract: Maternal cigarette smoking significantly increases the risk of serious pregnancy complications. Estimates suggest that each year tobacco use is responsible for 19,000 to 141,000 abortions, 32,000 to 61,000 low birth weight infants, 14,000 to 26,000 infants who require admission to neonatal intensive care units, and 1,900 to 4,800 infant deaths. Thus, it is not surprising that maternal cigarette smoking is unequivocally the largest and most important known, modifiable risk factor for low birth weight and infant death. In spite of the severe negative consequences of maternal smoking on pregnancy outcome, studies suggest that approximately 20-30% of pregnant women in this country smoke. Results of a recent population-based epidemiological study conducted in California highlight the problem locally. Prevalence of prenatal tobacco use was 9% in the general population, and 29% among mothers who did not receive prenatal care. It is important to note that in this study cigarette smoking was self-reported, i.e. not verified by biochemical testing. Thus, the actual rates of prenatal smoking are likely to be far greater. Currently, little is known about how maternal tobacco use is linked to

a dramatic increase in serious pregnancy complications. We are testing the theory that smoking harms the placenta. The placenta is a transient organ that exists only during pregnancy. Its short life span belies its unique functions which are vital to human development before birth. For example, pregnancy begins when placental cells attach the embryo to the mother's uterus. Once pregnancy is established, a child must develop in the uterus for many months before its organ systems can function on their own. During this time the placenta carries out, for the child, the roles played by many important organs including the heart, lungs, digestive system and kidneys. Thus, throughout this critical period toxic substances that harm the placenta likewise harm the developing child. Results of experiments we published during the previous grant period showed that the placenta is vulnerable to the toxic effects of maternal tobacco use. Smoking interferes with the way placental cells attach to the uterus, and subsequently, the way they function. Our findings suggest that the problems are primarily due to the negative effects of maternal smoking on placental growth, the focus of our current application. We envision that the results of our study could be used in cessation studies to help women quit smoking during pregnancy. Research suggests that pregnancy is an ideal time to intervene, since many women reduce or stop cigarette consumption on learning they are pregnant. However, the effectiveness of cessation programs in this population is greatly enhanced when they use materials that are specific to pregnant women. A simple explanation of how smoking harms a child before birth, one possible outcome of our work, could be an important part of these specially designed materials. It is likely that a subset of mothers who smoke during pregnancy will be more likely to quit if they better understand the added risk their infants incur as a result of their cigarette use. In addition, if the mother is able to stop smoking permanently, her own health will greatly benefit.

Title: Breast Cancer and the Environment on Long Island
Principal Investigator: Gammon, Marilie
Institution: University of North Carolina Chapel Hill, Chapel Hill, NC
Funding Agency: National Cancer Institute
Project ID: CA66572
Project Funding Period: 8 August 1995 – 31 July 2005

Abstract: This continuation proposes to follow-up 1,508 case women newly diagnosed with breast cancer who are participants in an ongoing population-based, case-control study of breast cancer among women on Long Island. The primary aims of the ongoing parent case-control study are to determine whether risk of developing breast cancer is increased among women with higher levels of serum organochlorine compounds, including DDT and PCBs, or higher levels of polycyclic aromatic hydrocarbons, assessed by PAH-DNA levels in blood samples. The proposed continuation will follow-up the case women 3- and 5-years after diagnosis of the primary breast cancer to identity environmental factors that affect the risk of disease-free and overall survival, including (1) serum levels of DDT and PCBs, and PAH-DNA adducts based on blood samples collected at the parent case-control interview; and (2) cigarette smoking, physical activity, hormone replacement therapy, changes in weight as an adult, alcohol diet, and other factors assessed by structured questionnaire during the parent case-control interview. An additional aim is to explore whether the survival risk associated with these potential environmental risk factors is modified by known prognostic indicators in the tumor, including p53 and HER-2/neu. During the 3- and 5-year follow-up periods, medical treatment and outcomes (recurrence, second primary) will be assessed by telephone interview with the subject and by checking with physicians and medical records. Mortality will be determined by cross-checking the National Death Index, and by contacting next of kin and physicians. For the parent study, blood samples were successfully obtained for 1,087 case women and assays of DDT/PCBs and PAH-DNA adducts will be completed as planned for 643 and 577 cases, respectively. Blood samples for the remaining 444 and 320 case women, respectively, with sufficient blood volume will be assayed for these environmental compound as part of the proposed study. Assays for HER2/neu overexpression in case tumor tissue, assessed by

immunohistochemisty, will be conducted for the proposed follow-up; funding for the immunohistochemical assays of p53 expression has already been obtained. Standard statistical techniques for the analysis of cohort data will be used to determine the risk of disease-free survival and overall survival, at the 3- and 5-year follow-up periods, associated with higher levels of environmental factors, with adjustments made for breast cancer treatment, breast tumor characteristics, and other clinical predictors of survival. Potential subgroup effects, with cases partitioned on p53 and HER-2/neu expression, the relation between environmental factors on breast cancer survival will be explored, where possible.

Title: Pilot—Prenatal Nicotine Exposure and the Dopaminergic System
Principal Investigator: Garcia-Davila, Martha I.
Institution: Georgetown University, Washington, DC
Funding Agency: National Cancer Institute
Project ID: CA084718-050004
Project Funding Period: Not available

Abstract: The mesolimbic system has been implicated in the addictive properties of most drugs of abuse and dependence including cocaine, heroin, amphetamines and nicotine from tobacco. Nicotine affects the dopaminergic system in multiple ways including increasing both the firing rate and bursting activity of dopaminergic cells and by enhancing synthesis, metabolism and release of dopamine. In these studies we propose to examine, using an animal model, the acute and chronic effects of nicotine of dopaminergic function (dopamine release and dopamine uptake) at various developmental ages. The relationship between the nicotine-induced change in nicotine acetylcholine receptor (nAChR) numbers following chronic prenatal nicotine and the number of dopamine transports will also be assessed. Finally, the identify of the nAChR involved in the dopaminergic regulation will be determined by labeling the receptors with [125I]epibatidine, a high affinity radioligand for a variety of nAChRs, and immunoprecipitation of the receptors with subunit-specific antibodies. To determine the identify of these nAChRs involved in regulating dopaminergic function may lead us to a better understanding of nicotine's effects in brain and in addictive properties during various developmental stages. This knowledge will also help in the development of specific therapeutics, as well as in assessing the validity of using nicotine as a therapeutic agent in developmental disorders or during pregnancy as an aid to quit smoking.

Title: Smoking and Lifestyle Risk Factors for Premenstrual Changes
Principal Investigator: Gold, Ellen
Institution: University of California, Davis, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 7RT-0105
Project Funding Period: 1 July 1998 – 31 December 2001

Abstract: Premenstrual symptoms are an important health problem among women of reproductive age. Approximately 50% of women experience premenstrual symptoms at some time in their lives. In this proposed project, we plan to investigate the relationship between smoking and premenstrual symptoms.

Active and passive smoking pose unique but preventable health risks to women. Exposure to tobacco smoke is known to affect reproductive hormones in women. Since these hormones are likely to play a role in causing premenstrual symptoms, it is plausible that smoking may increase the frequency and severity of symptoms. Some previous studies suggest that smoking may worsen premenstrual symptoms, however, results from other studies do not confirm these

findings. Passive smoking has not been investigated as a risk factor for premenstrual symptoms, but may also increase the frequency and severity of symptoms.

We plan to conduct a study that will examine the effects of active smoking and passive smoke exposure to premenstrual symptoms. Two groups of' women, between the ages of 20 and 39 will be studied to investigate the role of active and passive exposure to smoke and premenstrual symptoms. The first group of women will be recruited from Kaiser Permanente Medical Care Program in the Sacramento area. These women will be asked to participate in recording their symptoms and other habits and activities in a daily diary. Daily records over several menstrual cycles are important for accurate diagnosis of premenstrual symptoms. We will also conduct a secondary analysis of data previously collected in a study of reproductive health among women in the Semiconductor Health Study (SHS), including six premenstrual symptoms, daily smoking, passive smoke exposure and cotinine levels collected prospectively over a mean of 5 menstrual cycles.

A well-designed epidemiologic study of the effects of active and passive smoking on PMC will add much to our knowledge of the health consequences of active and passive exposure to smoke in reproductive age women. Additionally, the results will inform current prevention and treatment strategies for premenstrual symptoms.

Title: Biochemical Markers in the Nurses' Health Study Cohort
Principal Investigator: Hankinson, Susan
Institution: Brigham and Women's Hospital, Boston, MA
Funding Agency: National Cancer Institute
Project ID: CA49449
Project Funding Period: 1 January 1998 – 31 March 2004

Abstract: We propose to analyze blood samples in a nested case-control manner from the 32,826 participants in the Nurses' Health Study (NHS) who provided samples in 1989-90 and were 43 to 69 years of age at that time. The samples have been stored at greater than or equal to 130 C in liquid nitrogen freezers since collection. We will assay samples from women who were diagnosed after blood collection and matched controls who remained disease-free, thus efficiently utilizing these prospectively collected samples. We propose to build upon our recent positive findings for several plasma hormones and nutrients in relation to breast and colon cancer risk, and to address new hormonal hypotheses in relation to risk of breast, ovarian and colon cancers and myocardial infarction. Specifically, we will examine (1) plasma estrogens, androgens and prolactin levels in relation to breast cancer risk in postmenopausal women, (2) a polymorphism in the catechol-O- methyl transferase (COMT) gene and risk of breast cancer, (3) DHEA, DHEAS and 5-androstene-3beta,17beta-diol and risk of breast and ovarian cancers and myocardial infarction, (4) insulin-like growth factor I and its binding proteins 1 and 3, plasma vitamin D and polymorphisms in the vitamin D receptor, and plasma antioxidant levels - all in relation to risk of breast and colon cancers. The ongoing NHS (CA40356 and HL34594) will provide follow-up and documentation of disease outcomes in addition to providing information on important covariates (such as exogenous hormone use, diet, smoking status, among others) for the proposed study. Participation in the NHS has been high: among the 32,826 women who provided a blood sample, 98 percent continue to complete questionnaires, and vital status has been documented for 99 percent. Overall, the large size of the cohort, the prospective design, the high follow-up rate, the detailed exposure data, and the availability of archived blood specimens provide a unique opportunity to test several hypotheses of public health importance. We also propose to collect a second blood sample from about 18,000 of the women who provided a first blood sample in 1989-90. This will increase our statistical power in future analyses, and allow us to assess in detail the temporal relationships between biomarkers and disease risk and to reduce the attenuating effects of measurement error.

Title: Cigarette Smoking and Post-Partum Breast Cancer Risk
Principal Investigator: Hsieh, Chung-Cheng
Institution: University of Massachusetts Medical School, Worcester, MA
Funding Agency: National Cancer Institute
Project ID: CA88891
Project Funding Period: 13 September 2001 – 31 July 2003

Abstract: Cigarette smoking has been hypothesized to have both carcinogenic and anti-estrogenic effects that may offset each other to produce no overall effect on breast cancer risk. A full-term pregnancy also appears to have opposing effects on breast cancer risk: 1) an adverse effect shortly after delivery and 2) a beneficial effect over time. If the transient increase risk of breast cancer is due to the growth-enhancing consequences of elevated pregnancy hormones on already initiated cells, then cigarette smoking during pregnancy, through its anti-estrogenic effect, can be expected to dampen this risk. Conversely, with its carcinogenic effect, cigarette smoking during pregnancy might also reduce the long-term protection against breast cancer afforded by a full-term pregnancy. We propose to examine the effects of cigarette smoking on the risk of postpartum breast cancer occurring at different intervals following delivery. We will use a database that links together the Swedish Medical Birth Register, National Cancer Register, and Register of Causes of Death. Members of the study population are all mothers who delivered a liveborn or stillborn baby after a gestation period of at least 28 weeks in Sweden between 1973 and 1998. We have adopted a nested case-control sampling design to allow more efficient analyses. Cases are approximately 3,500 women who had one or more childbirths between 1973 and 1998 and who had a breast cancer diagnosis during the same period. For each case subject, five controls who were born in the same year as the index case, were alive at the date of the diagnosis for the index case, and had not been diagnosed with breast cancer by that date, will be randomly selected from the source population. Logistic regression analysis will be applied to examine cigarette smoking as a risk determinant for postpartum breast cancer adjusting for age, parity, and age at first full-term pregnancy.

Title: Maternal Caffeine Use and Pregnancy Outcome
Principal Investigator: Klebanoff, Mark A.
Institution: National Institute of Child Health and Human Development, Bethesda, MD
Funding Agency: National Institute of Child Health and Human Development
Project ID: HD002520
Project Funding Period: Not available

Abstract: The role of maternal caffeine consumption in the pathogenesis of adverse pregnancy outcomes is controversial. Several studies have found that women who consume caffeine are at increased risk of spontaneous abortion and fetal growth restriction compared to non-users. However, other equally well-done studies have found no harmful effects of caffeine consumption. In addition, several studies have reported that caffeine is harmful only among women who smoke. All previous studies of this question have relied on maternally-reported caffeine use; no studies have employed a biomarker for caffeine. This project first validated the use of serum caffeine and its metabolites as a marker for caffeine intake, and then studied these serum markers as a risk factor for adverse pregnancy outcome. In the validation study, serum paraxanthine was determined to be an acceptable marker for caffeine intake. As part of this project, the concentration of cotinine, a metabolite of nicotine, was assayed in the serum of approximately 450 of the women and the results compared to their reported cigarette smoking. Women were found to be very honest in reporting whether they smoked, but their serum concentration of cotinine correlated only moderately with the amount smoked. This was the case for two separate populations of women 30 years apart. In the main part of this project, the serum

concentration of paraxanthine, caffeine's primary metabolite, was compared between 591 women experiencing a spontaneous abortion and 2558 women with live births who had serum drawn at the same time of pregnancy as the women with spontaneous loss. In addition, the association between reduced fetal growth and third-trimester paraxanthine serum concentrations was evaluated among these 2515 women. The mean concentration of paraxanthine was higher in women experiencing a spontaneous abortion than women experiencing a live birth (752 vs 583 ng/ml). Further analysis revealed that this may be explained by a few women with very high concentrations of paraxanthine. The odds ratio for serum paraxanthine concentration greater than the 95th percentile was 1.86 ($p<0.01$), but intermediate concentrations were not associated with an elevated risk of spontaneous abortion. These results suggest that moderate caffeine consumption during pregnancy does not increase the risk of spontaneous abortion. Among the control women, 2515 provided a third-trimester serum sample. The mean paraxanthine concentration in this sample was higher among women who subsequently gave birth to a small-for-gestational age (SGA) infant (754 ng/ml) than among women who gave birth to appropriately-grown infants (654 ng/ml, $p=0.03$). There was no statistically significant association between paraxanthine and SGA birth among non-smokers ($p=0.48$). Among smokers, increasing serum paraxanthine concentration was associated with an increased risk of SGA birth ($p=0.03$).

Title: Smoking, Estrogen Metabolites & Cognitive Function
Principal Investigator: Kritz-Silverstein, Donna
Institution: University of California, San Diego, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 9RT-0034
Project Funding Period: 1 July 2000 – 30 June 2002

Abstract: Previous studies reported that cigarette smoking is associated with lower levels of endogenous estrogen in the blood and an earlier age at menopause. Smoking has also been associated with poorer cognitive function. However, the literature has not reported on the association between smoking, estrogen and cognitive function, and the information that is available on estrogen and cognitive function is inconsistent. This may be because smoking alters the breakdown of estrogen in the body causing more of it to be converted to an inactive form. If estrogen is protective against memory loss and impaired cognitive function, smoking would have an effect of decreasing cognitive function via the estrogen metabolism pathway. This will be the first study to measure estrogen metabolites in a large sample of women aged 65 and older. It will determine if smoking is related to cognitive function at baseline and to change in cognitive function over time through alterations in estrogen metabolism. We will also look at the effects of smoking and estrogen metabolism in women who develop Alzheimer's disease. Results of this study will help elucidate the mechanism by which smoking exerts an effect on a common disease and will provide further evidence that Alzheimer's disease and cognitive decline is a tobacco-related disease.

Title: Transport and Disposition of Nicotine in the Human Placenta
Principal Investigator: Kroetz, Deanna
Institution: University of California, San Francisco, San Francisco, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 7RT-0025
Project Funding Period: 1 July 1998 – 30 June 2002

Abstract: Smoking during pregnancy is associated with many adverse outcomes on both the pregnant woman and on her fetus and newborn baby. Some of these effects include an increased chance of premature birth, a higher rate of infant death, a slower rate of fetal growth, low birth

weight of the newborn, and an increased incidence of sudden infant death syndrome. Most of these adverse effects are associated with nicotine, a major component of tobacco smoke. Nicotine is also responsible for the addictive properties of this drug. With at least 16% of women continuing to smoke throughout their pregnancy, the effects of smoking on the pregnant woman and fetus are major public health concerns. This study will investigate if and how the placenta protects the fetus from nicotine. The placenta serves as a major life support organ for the fetus. Its ability to move oxygen and nutrients from maternal to fetal blood is necessary for the normal growth and development of the fetus. It also serves as a protective barrier for the fetus against harmful compounds by preventing their passage from maternal blood. Since nicotine is such a commonly used and abused drug during pregnancy, it is important to understand how the placenta functions to prevent nicotine from reaching the fetus. Specifically, this project will examine the mechanisms by which nicotine moves across cell membranes, allowing its entry both into and out of the fetus and placenta. It is likely that, once nicotine enters the placenta, it encounters "pumps" which return the nicotine back to the maternal blood, thereby protecting the fetus. We will also test whether the placenta can break down nicotine into less harmful agents. This is another possible way in which the placenta can protect the fetus from the harmful effects of nicotine (and other drugs and toxins). These studies will also determine whether the placentas of smoking women are better able to pump nicotine away from the fetus and to break nicotine down into less harmful compounds in comparison to the placentas of non-smoking women. This would mean that the placenta may have important ways in which it responds to not only nicotine but also other potentially harmful drugs as a means of protecting the fetus. Overall, these studies will greatly increase our understanding of the role of the placenta in regulating the exposure of the fetus to nicotine. This information is vitally important for us to decrease the health risks of smoking women and their newborn infants.

Title: Smoking & Genetic Interaction in Breast Cancer Etiology
Principal Investigator: Lash, Timothy L.
Institution: Boston University, Boston, MA
Funding Agency: National Cancer Institute
Project ID: CA087724
Project Funding Period: 4 August 2000 – 31 July 2004

Abstract: The candidate, Dr. Timothy Lash, has been committed to cancer research since the beginning of his career. He studied molecular biology, including tumor biology, as an undergraduate at the Massachusetts Institute of Technology. Upon graduating, he worked as a consultant in environmental health on projects that required dose-response assessment of environmental and occupational carcinogens. Simultaneously, he completed a Masters of Public Health and the coursework for a Doctorate of Science in Epidemiology at the Boston University School of Public Health. The curricula for these degrees included many courses directly relevant to cancer prevention and control. He has worked on research projects involving breast cancer etiology, therapy, and side-effects of therapy under the direction of the proposed mentor (Dr. Rebecca Silliman) and the proposed co-mentor (Dr. Ann Aschengrau). Dr. Lash is currently an Assistant Professor of Epidemiology at the Boston University School of Public Health. He spends 75 percent of his effort on research projects directly relevant to the proposed research plan. For example, he is the project director on a study of adjuvant tamoxifen therapy in old age. Dr. Silliman is the Principal Investigator of the project, which will provide the cohort of breast cancer patients eligible for the second proposed study. Dr. Lash also works with Dr. Aschengrau, the proposed co-mentor, on analyses of the effect of active and passive smoking on breast cancer occurrence. The most recent case-control data set in which these analyses have been performed will provide the subjects eligible for the first proposed study. The environment at the Boston University Medical Center is ideally suited for accomplishing the career development goals of this application. The research plan proposes two studies of the interaction between tobacco

smoke and NAT2 or COMT genetic polymorphisms. The association between tobacco smoke and breast cancer risk is complicated. It has been hypothesized that tobacco smoke exposure may both cause and prevent breast cancer, depending on the timing of exposure relative to reproductive milestones. The interaction with tobacco smoke exposure with the gene polymorphisms will allow tests of these hypotheses. The first study would collect buccal swabs from eligible participants of the second Cape Cod case-control study (Dr. Aschengrau was PI). Existing interview information would be combined with the polymorphism data extracted from the swabs to assess the interaction between the polymorphisms and tobacco smoke subgroups under a biologically based etiologic model of breast carcinogenesis. The second study would collect buccal swabs from participants in the study of adjuvant tamoxifen therapy (Dr. Silliman PI). A similar analytic plan would be conducted, but with a case-only design.

Title: Effects of Smokeless Tobacco Use on Pregnancy
Principal Investigator: Levine, Richard J.
Institution: National Institute of Child Health and Human Development, Bethesda, MD
Funding Agency: National Institute of Child Health and Human Development
Project ID: HD008745
Project Funding Period: Not available

Abstract: Smoking cigarettes during pregnancy adversely affects pregnancy outcomes. Smokeless tobacco is thought to be a safer alternative to smoking because combustion products are not generated. We are studying the effects of smokeless tobacco on pregnancy outcomes in a retrospective cohort study of women in the Swedish Medical Birth Register. We will compare pregnancy outcomes of those who used snuff daily, but did not smoke cigarettes; those who smoked cigarettes daily, but did not use snuff; and those who used neither product. Associations between tobacco exposure and birth weight, preterm delivery, and preeclampsia will be examined.

Title: Modeling the Relation of Smoking to the Ovarian Function
Principal Investigator: Liu, Yan
Institution: University of California, Davis, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 8DT-0172
Project Funding Period: 1 January 2000 – 31 January 2002

Abstract: Other than its well-known carcinogenic effect, tobacco smoke might also act as a toxin to the reproductive endocrine environment in women. Menstruation and ovarian steroid patterns are primary markers of human female reproductive endocrinologic and ovarian function. Researches only involve laboratory animals or a few women have show that tobacco smoke could influence ovarian sex hormone biosynthesis. Investigation of menstruation and its hormonal bases based on large-sample-size data is limited. In fact, major difficulties in analysis and interpretation of cyclic menstrual and hormone data arise when much of the observed variability in menstrual characteristics stems from changes within women as opposed to differences between women and steroid data are curved over an entire menstrual cycle.
The improvement of less-cost biological assay techniques, rapid development of computer hardware/software and considerable exploration of modern statistical inference methods allow us to model human ovarian function based on cycle-to-cycle daily diary and urine collection now. The primary goal of the research proposed is to explore methodology for investigating the influence of cigarette smoking in the presence of other host and environmental factors on the ovarian function of women based on daily diary and urinary assay data. The study data were collected previously. The importance of this existing database is that it contains information on urine specimens collected not only over an entire menstrual cycle for each of 402 women but

also over several successive cycles for most of those women. The effects of cigarette smoking on ovarian steroid secretion and metabolic clearance rate (MCR) of progesterone, in premenopausal women will be assessed with control of variables we collected, such as age, race, parity, body mass index (BMI), alcohol use, caffeine consumption and physical activity. We also want to evaluate the relation of menstrual cycle length and follicular and luteal phase lengths with tobacco exposure.

The response variables from an individual are usually correlated since they were measured repeatedly over time. In the present proposed study, appropriate models that allow for such correlation is discussed. Menstrual characteristics will be modeled as linear functions of the smoking and other variables using the general linear mixed model (GLMM). But typical menstrual cycle measures may not be sensitive indicators of changes or disturbances in hormonal function resulting from cigarette smoke exposure. We will model curves which reflect hormone profiles over time using variants of the GLMM, which are expected to be more sensitive to such changes. The above analyses rely on the assumption of normality of the data and on having moderate to large sample sizes. We will perform a special technique, bootstrap analysis, in order to check on these assumptions and to provide inferences that are less dependent on these assumptions.

The present proposed study should shed light on the mechanisms by which cigarette smoke adversely affects women's endocrinologic and menstrual function and provide new approaches for scientists in their efforts to detect more sensitively the tobacco-related health effects with respect to reproduction in premenopausal women.

Title: Relationships Between Smoking, Homocysteine & Folate
Principal Investigator: McEligot, Archana
Institution: University of California, San Diego, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 9DT-0046
Project Funding Period: 1 July 2000 – 30 June 2002

Abstract: The relationship between cigarette smoking and increased risk of cardiovascular disease (CVD) has been well established. Studying the biological link between smoking and CVD needs further investigation. Increased blood levels of homocysteine may increase risks of heart disease. Homocysteine is an amino acid, and consuming a diet high in meat and dairy products has been shown to increase blood homocysteine levels. Studies have suggested that smokers have higher blood levels of homocysteine than non-smokers and the levels of homocysteine increase with the number of cigarettes smoked. Homocysteine levels can be lowered by consuming three vitamins: folate (mostly found in fruits and vegetables), vitamin B-12 and vitamin B-6. Folate, in particular, has been shown to substantially reduce homocysteine levels. Some studies that have reported on the link between cigarette smoking and homocysteine have not examined folate levels in smokers and non-smokers, which could alter the results. Also most studies use folate from vitamin supplements rather than from fruit and vegetables to lower homocysteine levels, and therefore the effects of consuming a high vegetable diet on homocysteine concentrations have not been widely investigated. We would like to examine blood homocysteine and folate levels in a group of female smokers participating in a randomized clinical trial. The randomized trial is investigating the effects of consuming a diet low in fat and high in vegetable, fruit and fiber on breast cancer recurrence. We will examine homocysteine and folate levels upon entry into the study and one-year after randomization into either the intensive diet group (high vegetable and fruit diet) or the control group (usual diet). We expect that the smokers in the intensive diet group will have lower homocysteine concentrations than the control group at one-year post-randomization, which will show that a high vegetable diet may be protective for smokers by reducing homocysteine. Also, we will be

able to compare homocysteine levels in smokers and non-smokers at entry into the study and one-year post-randomization. If smokers have higher amounts of homocysteine concentrations than non-smokers, regardless of their folate levels, then we could hypothesize on a possible mechanism for higher CVD rates in smokers. Thus, reducing homocysteine levels in smokers and investigating possible explanations for higher rates of heart disease in this population may provide future avenues of preventing or curbing the risk for CVD in smokers.

Title: Nicotine Effects on Neurological Development
Principal Investigator: Metherate, Raju
Institution: University of California, Irvine, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 8RT-0059
Project Funding Period: 1 July 1999 – 30 June 2002

Abstract: A particularly tragic effect of tobacco smoke is what it does to unborn and newborn babies, including how it affects their brain growth. Nicotine in a mother's bloodstream can cross the placenta to affect brain development in the fetus. Even after birth, nicotine in secondhand smoke inhaled by infants can affect their growing brains. For example, studies have shown that smoking by pregnant mothers results in babies that do not respond as well as they should to sounds around them. As a result, their abilities later on in life to hear and speak normally are hurt. Since these babies have normal input from their ears to their brain, their problems come from improper brain development. Similar problems also occur in infants who inhale secondhand smoke. It is not known why nicotine has this effect, nor is it known how much nicotine must be inhaled in order to produce these effects. This research will determine how exposure to nicotine affects the normal development of the auditory cortex (the highest brain center responsible for hearing). Scientists think that a protein called the NMDA receptor is important for the development of the auditory cortex (and similar areas for sight and touch). Recent studies have shown that nicotine affects the normal functions of the NMDA receptor. The effect is strongest during a brief period of time shortly after birth. This may be the time when the growing brain is most vulnerable to the effects of tobacco smoke. By placing tiny electrodes into brain cells to measure their activity, we will determine exactly how nicotine affects the NMDA receptor. We will also determine how much exposure to nicotine is sufficient to affect brain growth. Finally, we will test auditory functions in adult rats that were exposed to nicotine as pups, to determine how exposing babies to nicotine will affect their abilities as they age. This research will benefit the public by increasing our understanding of how nicotine affects brain function.

Title: Metabolic Genotypes & Oncogenic Damage in Breast Cancer
Principal Investigator: Miller, Mark
Institution: Wake Forest University, Winston-Salem, NC
Funding Agency: National Cancer Institute
Project ID: CA81330
Project Funding Period: 1 July 2000 – 30 June 2005

Abstract: Interactions between environmental and genetic factors have been implicated in the etiology of breast cancer. In particular, several studies have suggested that the type of genetic damage observed in human breast tumors may be influenced by exposure to chemical toxicants. However, few attempts have been made to compare the ability of breast tissue to metabolize chemical carcinogens with the types of mutations observed at critical oncogenic loci. To better understand the etiology of breast carcinogenesis and determine the role of gene/environmental interactions in determining individual susceptibility to breast cancer formation, a prospective case-case study design will be utilized to compare mutations in the p53 gene with the genotype

of affected cancer patients for 4 metabolic enzymes (CYP1A1, GSTM, GSTT, and GSTP) that play key roles in the metabolism of human environmental carcinogens. We hypothesize that those breast cancer patients containing either specific alleles of CYP1A1 that enhance the metabolic activation of environmental toxicants or genotypes of GSTs that would result in less detoxification will be more likely to have accrued genetic damage at the p53 locus, and that combinations of alleles that increase the burden of reactive electrophiles will be more susceptible to tumor initiation. Tumor tissue samples will be analyzed for genetic alterations in p53 by SSCP and gene sequencing analyses. DNA obtained from blood will be genotyped by PCR-RFLP to determine if patients harboring genetic damage to p53 more frequently exhibit metabolic genotypes that increase formation of reactive electrophiles from environmental toxicants. A prospective study design will allow use of a questionnaire to identify other potential factors (including smoking, diet, occupation, race, and reproductive history) that may modify the association between genotype and mutations to p53. As mutation at p53 has been implicated in poor patient prognosis, these studies should aid in identifying those patients at risk for damage to key regulatory genes that play a role in the pathogenesis of breast cancer, and will further our understanding of the etiology and risk factors for this disease.

Title: Smoking During Pregnancy and Breast Cancer
Principal Investigator: Mueller, Beth A.
Institution: Fred Hutchinson Cancer Research Center, Seattle, WA
Funding Agency: National Cancer Institute
Project ID: CA096434
Project Funding Period: 1 April 2002 – 31 March 2004

Abstract: Although cigarette smoking has been linked to the etiology of several cancers, the relationship between smoking and breast cancer remains unclear. A woman's first pregnancy represents a period of rapid breast cell growth and differentiation and thus, a period of vulnerability to the influences of smoking or other exposures. During pregnancy, tobacco mutagens and free radical formation caused by smoking may affect rapidly growing breast tissue or act synergistically with elevated estrogens to increase breast cancer risk. Because breast tissue is less differentiated at the onset of first pregnancy, it may be more susceptible to mutagenesis than in subsequent pregnancies. The proposed population-based case-control study will utilize linked vital statistics - cancer registry data to test the hypothesis that cigarette smoking during first pregnancy is related to the risk of breast cancer. The specific aims of this study are to: 1)measure the risk of breast cancer associated with smoking during a first pregnancy relative to not smoking during the first pregnancy, and 2)evaluate possible differences in the relation between smoking during first pregnancy and breast cancer by tumor estrogen receptor status. To the extent possible, the study will also evaluate a possible dose response relation between the average number of cigarettes smoked per day during first pregnancy and breast cancer risk, and measure possible differences in the relation between smoking during first pregnancy and breast cancer risk by subject characteristics such as parity at the time of diagnosis and pre-pregnancy weight. This study will be among the first to examine smoking during first pregnancy and breast cancer risk. The clarification of the role of smoking during first pregnancy in breast cancer development will aid in understanding the complex etiology of breast cancer, and may identify a specific preventive strategy to help reduce breast cancer incidence.

Title: Effect of Cigarette Smoking on Pulmonary Metastasis
Principal Investigator: Murin, Susan
Institution: University of California, Davis, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 10IT-0264
Project Funding Period: 1 July 2001 – 30 June 2002

Abstract: Smokers are more likely to die of breast cancer than are non-smokers, though they don't get breast cancer any more often. This suggests that breast cancer may behave more aggressively among smokers. The reasons for this are not clear. Studies with animals have shown that a variety of things that injure the lung, like exposure to high concentrations of oxygen or to radiation, do increase metastasis to the lung. Because smoking also injures the lung, we think it may make breast cancer more likely to spread to the lung, but this has not been directly studied. Studies in populations of patients have suggested that smoking may encourage the spread of breast cancer to the lungs, but these types of studies can only indirectly look at the association between smoking and breast cancer behavior. We propose to examine the relationship between smoking and the spread of breast cancer to the lungs more directly, in an animal model of breast cancer. We will study the effect of smoking on the spread of breast cancer to the lung by measuring the number and size of breast cancer deposits in the lung among mice exposed to cigarette smoke compared to mice not exposed to cigarette smoke. The experiments will be carried out using specialized smoke-exposure chambers that generate smoke concentrations that are comparable to those experienced by actively smoking adults. The breast cancer model in the mice is one that is very much like the human situation and that has previously been used to answer other questions about things that affect the spread of breast cancer, such as diet. We will compare both smoking and non-smoking animals, as well as a third group of animals that stops smoking after the breast cancer has grown for awhile. We include this third group because it allows us to model the situation of a woman quitting smoking at the time her breast cancer is diagnosed. If smoking does cause breast cancer to spread more easily, it will be important to see if this effect can be prevented by a woman quitting smoking when she finds out about her cancer. Since breast cancer and smoking are both very common among women an effect of smoking on the behavior of breast cancer is potentially quite important. This is especially true if the adverse effect of smoking can be reversed by quitting smoking at the time of breast cancer diagnosis. Even if it can not be reversed, it is still important. Women smokers may be persuaded to quit smoking if they know it will make them more likely to die should they get breast cancer. Also, studies that compare women with breast cancer will need to take their smoking status into account if smoking really does make a difference in the way breast cancer behaves.

Title: Active and Passive Tobacco Smoke Exposure, NAT2 Genotype, & Breast Cancer Risk
Principal Investigator: Newschaffer, Craig J.
Institution: Johns Hopkins University, Baltimore, MD
Funding Agency: American Cancer Society
Project ID: RPG-99-031-01-CCE
Project Funding Period: January 1999 – December 2002

Abstract: This project will study a possible link between smoking and breast cancer. It will determine whether smoking and being exposed to other people's smoke increases certain women's risk of breast cancer. Although we already know that smoking causes other cancers, we also know that about 23 million women are still smoking. However, if there is a link with breast cancer then new and powerful anti-smoking messages aimed toward women could be developed. The study will also help scientists better understand the complicated process behind cancer. As with other cancers, many different courses contribute to breast cancer with some being more important in different women. Understanding which individuals are threatened most by different

risk factors is one of the keys to further unraveling the puzzle of cancer. This study is important because it will determine if there is a group of women who, because of differences in the way their bodies handle certain substances in tobacco smoke, are placed at a higher risk of breast cancer when exposed to smoke.

Title: Effects of Maternal Smoking on Fetal Catecholamine Secretion During B
Principal Investigator: Oncken, Cheryl
Institution: University of Connecticut School of Medicine and Dentistry, Farmington, CT
Funding Agency: National Center for Research Resources
Project ID: RR006192-090156
Project Funding Period: Not available

Abstract: Maternal smoking is the most preventable cause of poor pregnancy outcomes in the United States. This study will provide useful information on potential mechanisms by which maternal smoking may contribute to SIDS, and may provide essential data for future work in detection and prevention of tobacco-related disturbances. This study utilizes a between-subject design to compare the effects of prenatal smoking on catecholamine response to hypoxia during delivery. Forty women will be identified as smokers or non-smoking (20 per group) at 28 weeks gestational visit.

Title: Effects of Nicotine on Bone Turnover in Older Women
Principal Investigator: Oncken, Cheryl
Institution: University of Connecticut School of Medicine and Dentistry, Farmington, CT
Funding Agency: National Center for Research Resources
Project ID: RR006192-070193
Project Funding Period: 15 December 1993 – 30 November 2003

Abstract: We have begun a study to examine the effects of nicotine on bone turnover, largely based on the preliminary results of our work showing that smoking cessation reduces markers of bone resorption by 20%.

Title: Research on Maternal Depressive Symptoms and Postpartum Smoking
Principal Investigator: Orr, Suezanne
Institution: East Carolina University School of Health and Human Performance, Greenville, NC
Funding Agency: Robert Wood Johnson Foundation
Project ID: 043281
Project Funding Period: 1 March 2002 – 29 February 2004

Abstract: This grant provides supplemental funding for research focused on the relationships between maternal prenatal depressive symptoms (and other psychosocial factors) and maternal prenatal smoking cessation and intensity (i.e., number of cigarettes smoked). During the current study, data are being collected on psychosocial factors and smoking status at the first prenatal visit and at 30 weeks gestation. During this study, the research team will collect follow-up data at the 6- or 12-week postpartum visit and again at 6 to 12 months postpartum. The purpose of this further research is allowing the team to thoroughly measure smoking status during the postpartum period, which will greatly aid the team's findings. The findings from this research will lead to future new treatments of depressive symptoms as a way to prevent smoking relapse and facilitate smoking cessation.

Title: Vaccine Effects on Fetal Nicotine Exposure in Rats
Principal Investigator: Pentel, Paul R.
Institution: Minneapolis Medical Research FDN, Inc., Minneapolis, MN
Funding Agency: National Institute on Drug Abuse
Project ID: DA015668
Project Funding Period: 30 September 2002 – 31 July 2006

Abstract: The aim of this proposal is to study the effects of maternal immunization with a nicotine vaccine on the pharmacokinetics, neurochemical consequences, and behavioral sequelae of gestationally administered nicotine in rats. Smoking during pregnancy is associated with a wide range adverse neonatal outcomes. Animal data strongly implicate nicotine as a teratogen and a contributor to these adverse outcomes. It has recently been shown that immunization of adult male rats with a nicotine vaccine can substantially reduce the distribution of acutely or chronically administered nicotine to brain and other organs. Preliminary data suggest that vaccination of female rats can also reduce the distribution of gestationally administered nicotine to fetal brain. The proposed study will address the mechanisms by which vaccination alters nicotine distribution to the fetus, and the magnitude and consequences of this effect under a variety of dosing conditions. The pharmacokinetics of nicotine in the pregnant rat will also be studied to better understand the determinants of fetal nicotine exposure. Specific hypotheses to be tested are that 1) Maternal vaccination reduces the distribution to fetal brain of nicotine administered during gestation using a variety of clinically relevant acute and chronic dosing regimens. 2) Protection of fetal brain from gestational nicotine exposure occurs via two complementary mechanisms; a reduction in unbound nicotine distribution to the fetus, and the transfer of maternal antibody to the fetus which then binds and sequesters nicotine in fetal serum, 3) Vaccination attenuates the increase in fetal brain c-fos mRNA expression associated with chronic gestational nicotine exposure, 4) Vaccination attenuates the increase in neonatal locomotor activity associated with gestational nicotine exposure, and 5) Nicotine clearance is lower in nonpregnant females than in males, but is increased in females during pregnancy. These immunologic, pharmacokinetic, neurochemical and behavioral data will be integrated to help understand the mechanisms by which vaccination alters fetal nicotine distribution, and the clinical potential of vaccination to reduce the teratogenic effects of gestational nicotine exposure.

Title: Health Effects of PAH & ETS in Minority Women & Newborns
Principal Investigator: Perera, Frederica P.
Institution: Columbia University Health Sciences OGC, New York, NY
Funding Agency: National Institute of Environmental Health Sciences
Project ID: ES008977
Project Funding Period: 1 August 1997 – 31 July 2002

Abstract: There is increasing evidence that people of color are disproportionately exposed to numerous environmental hazards, including hazardous air pollutants such as polycyclic aromatic hydrocarbons (PAH) and environmental tobacco smoke (ETS). The Washington Heights and Harlem neighborhoods in Manhattan are typical of other Hispanic and African American communities in that they are located in a large sprawling metropolitan area characterized by elevated air pollution. The incidence of low birth weight is higher among African Americans living in Central Harlem and Hispanics living in Washington Heights than in Caucasians in the U.S. Cancer rates are also higher in African Americans than in Caucasians. Environmental risks to the developing infant are of particular concern, given the likelihood of increased susceptibility during this period. A molecular epidemiologic cohort study of African American and Hispanic mothers and newborns is proposed to investigate the role of PAH and ETS in procarcinogenic and developmental damage. A combination of personal monitoring, questionnaire and biomarkers in peripheral blood will be used to quantify individual exposure to the toxicants of concern. The biomarkers include PAH-DNA adducts in white blood cells (an indicator of PAH

exposure and procarcinogenic genetic damage) and plasma cotinine (a metabolite of nicotine and internal dosimeter of ETS). Measures of development will be assessed in the infants at birth and at 6 and 12 months. The proposal is responsive to concerns about environmental justice and to the recommendation of the National Research Council that risk assessment and public health policy pay special attention to the protection of young infants and children.

Title: Environmental Influences on Perinatal Lung Development
Principal Investigator: Pinkerton, Kent
Institution: University of California Davis, Davis, CA
Funding Agency: National Institute of Environmental Health Sciences
Project ID: ES011634
Project Funding Period: 1 May 2002 – 28 February 2007

Abstract: Childhood exposure to a variety of indoor air contaminants including environmental tobacco smoke (ETS) produces significant risks in asthma, airway hyperresponsiveness, and other respiratory symptoms such as cough, wheeze, and mucus production. Epidemiological studies suggest that exposure to ETS during the perinatal period may have adverse effects on lung function which can persist into adulthood. It has been estimated in the United States alone 200,000 to 1,000,000 children with asthma will have their condition worsened by exposure to ETS (USEPA, 1992). However, the mechanisms leading to this process are unknown. During the past 2.5 years, we have established a state-of-the-art inhalation system to study the effects of exposure to ETS on perinatal lung development in a nonhuman primate, the Rhesus macaque monkey. Exposure to ETS has been done using aged and diluted sidestream cigarette smoke as a surrogate for ETS. Two chambers located at the California Regional Primate Research Center Inhalation Facility have been expressly designed and configured to create conditions for passive smoke exposure to monkeys during pregnancy and early postnatal development under carefully controlled conditions. These studies have demonstrated significant alterations in lung development following exposure to ETS. These effects include changes in immune effector and inflammatory cells in the lung air spaces, alterations in pulmonary and peripheral blood cytokines and neurotrophins, alterations in the innervation and epithelial composition of the trachea, and changes in the activity and distribution of pulmonary cytochrome P450 monooxygenases and glutathione-S-transferases. All these changes are evident by 2.5 months of age in infant Rhesus monkeys. These findings confirm that ETS exposure during perinatal development significantly affects the lungs of non-human primate infants. We hypothesize that these changes represent the initial steps in the genesis of an asthmatic-like condition solely due to perinatal exposure to ETS. We also hypothesize that critical windows of exposure are present during the perinatal period which will exacerbate this effect. We propose to test these hypotheses by continuing to study this model in the Rhesus monkey to (1) determine the effects of exposure to ETS during specific periods of perinatal development in monkeys from early gestation to 6 months postnatal age using physiologic, biochemical and anatomical measures and (2) determine if cessation of exposure to ETS following 6 months postnatal age will still be associated with persistent changes in the respiratory system. Such studies should help us to better understand the potential mechanisms by which fetal and early postnatal exposure to environmental contaminants could lead to lasting, adverse consequences in children.

Title: Environmental Tobacco Smoke and Newborn Lung Development
Principal Investigator: Pinkerton, Kent
Institution: University of California Davis, Davis, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 6RT-0327
Project Funding Period: 1 July 1997 – 30 June 2001

Abstract: Environmental tobacco smoke (ETS) is defined as the combination of sidestream smoke released from the burning end of a cigarette and that portion of mainstream smoke exhaled by smokers. Health effects associated with exposure to ETS have been clearly demonstrated, but still remain somewhat controversial. In 1993 the U.S. Environmental Protection Agency declared environmental tobacco smoke (ETS) to be a human carcinogen that kills approximately 3,000 nonsmokers a year. Young children are also affected by ETS exposure with increases in the incidence of pneumonia, bronchitis, and middle ear infection. For children with asthma, ETS exposure increases the severity and frequency of asthmatic attacks. However, the cause(s) for greater and more adverse health risks associated with ETS exposure in children compared with adults is (are) unknown.

We propose to study in our laboratory the effects of exposure to ETS on the maturation and function of the lung airways during fetal and early postnatal development in rats. This species is ideal to study due to its rapid fetal and postnatal growth to adulthood over a period of approximately 70 days. We have found that ETS exposure during the fetal and early postnatal periods is associated with a significant increase in lung airway narrowing, similar to that seen in asthmatic children. Also associated with these hyperactive airways is a dramatic increase in pulmonary neuroendocrine cells (PNECs), which compose a small fraction of the cells lining the lung airways. The function of PNECs is unknown; however, they are thought to be important in the pulmonary response to substances that are inhaled into the lungs and help to regulate airway tone and function. It is our hypothesis that PNECs may be directly responsible for increased airway responsiveness (such as that seen in asthma) by either releasing bronchoconstrictive mediators or by increasing the mass of airway smooth muscle through smooth muscle cell proliferation. We propose to test this hypothesis in rats using environmental tobacco smoke (ETS) with exposure occurring during critical periods of lung growth and development from fetal to early adulthood in the rat. An important observation from our laboratory has been that when rats are exposed to ETS in utero (via the mother) followed by direct exposure for the first 3 weeks of life, their lungs remain markedly hyperresponsive when measured at 8 weeks of age, despite the absence of ETS exposure for the preceding 5 weeks. The discovery of this change in the lungs due to smoke exposure in newborn rats is highly analogous to the development of childhood asthma in humans. This animal model affords us the opportunity to examine whether there is a critical period during lung development when exposure to ETS leads to increased airway hyperresponsiveness and whether changes in airway PNEC number and/or function might be responsible for these observed changes.

Title: Developmental Exposure to Nicotine
Principal Investigator: Poland, Russell E.
Institution: Cedars Sinai Medical Center, Los Angeles, CA
Funding Agency: National Institute on Drug Abuse
Project ID: DA014680
Project Funding Period: 30 September 2001 – 31 July 2004

Abstract: This is an R21 application to explore the development of an animal model to study the effects of parental and peripuberal exposure to nicotine. Nicotine remains an important drug of abuse worldwide. In the United States (U.S.), tobacco use is the single leading preventable cause of death. However, despite considerable negative publicity and health warnings,

approximately 25 percent of the U.S. population still smoke. Aside from producing profound behavioral effects in the adult organism, nicotine also disrupts developmental processes in many species. Recent epidemiologic data suggest that fetal exposure to nicotine increases the risk for tobacco use during adolescence and adulthood, particularly in females. In addition, 75 percent of adult tobacco users report their first tobacco use occurred when they were "youngsters" (childhood or adolescence). In order to study this issue further, as well as to develop an animal model to elucidate potential underlying mechanisms, the effects of nicotine exposure during gestation on nicotine self-administration in adult male and female rat offspring will be studied. In addition, the effects of nicotine exposure during the periadolescent period on nicotine self-administration in adult offspring will be ascertained. It is hypothesized that nicotine exposure in utero will increase nicotine self-administration in adult offspring. Similarly, peripuberal exposure to nicotine also will increase nicotine self-administration during adulthood. The proposed studies will characterize the relationships between exposure to nicotine during critical periods of development and the acquisition, maintenance, extinction and re-initiation phases of nicotine self-administration. The results of these experiments should provide new and important insights on the relationships between prior nicotine exposure and nicotine-seeking behavior. In addition, since nicotine is considered as a "gateway" drug for the subsequent use of alcohol and other illicit drugs, the results of the proposed studies will lay the groundwork to further understand the factors which might increase vulnerability to drug addictions in general, and to nicotine abuse, in particular.

Title: Susceptibility To Molecular Alteration: Epithelial Cells
Principal Investigator: Powell, Charles A.
Institution: Columbia University Health Sciences Ogc, New York, Ny
Funding Agency: National Institute of Environmental Health Sciences
Project ID: ES000354
Project Funding Period: 26 September 1999 – 31 August 2004

Abstract: The objectives for this K23 Mentored Patient Oriented Research Award are to acquire conceptual and technical skills in cancer genetics, molecular epidemiology, and patient oriented research and subsequently to apply these skills to improve the survival of patients with lung cancer or at risk for developing lung cancer. These goals will be achieved through a comprehensive program that includes formal education, patient oriented research and clinical activities. The educational program will include courses on molecular genetics, clinical epidemiology and clinical research. Regular participation in national meetings and symposia related to cancer and molecular genetics will be an essential part of the educational program. The clinical activities will be focused on lung cancer. The long-term objective for the patient oriented research project is to identify the environmental and genetic factors that are responsible for the increased incidence of lung cancer in women. Recent statistical trends and case-control studies suggest that women are more susceptible to lung cancer than men with similar tobacco exposure. The goal for this research project is to determine if biomarkers of DNA exposure to carcinogens (DNA adducts) and biomarkers of effect (loss of heterozygosity and p53 mutations) in epithelial cells are involved in lung cancer susceptibility in women. The specific aims of the project are to determine if the extent of epithelial cell DNA adducts, LOH, and p53 mutations in women is different than in men with similar tobacco exposure and to determine the effect of gender, genetic polymorphisms, and diet on the relationships among these biomarkers of exposure and biomarkers of effect. This study will evaluate 60 female and 60 male middle aged smokers. Biomarkers will be evaluated in epithelial cells from the lung, bladder and oropharynx from all individuals. Polymorphisms of CYP1A1 and GSTM1 and dietary intake of vitamins and carotenoids will be characterized. These experiments will provide important information on how genetic and environmental factors are involved in lung cancer susceptibility in women. The

definition of these mechanisms may allow the prospective identification of individuals at increased risk for developing lung carcinoma.

Title: Adverse Pregnancy Outcomes: Genetic/Environmental Causes
Principal Investigator: Schatten, Gerald P.
Institution: Magee Womens Health Corporation, Pittsburgh, PA
Funding Agency: National Institute of Environmental Health Sciences
Project ID: ES012359
Project Funding Period: 30 September 2002 – 31 July 2007

Abstract: Life begins in utero typically. Prenatal environmental exposures, coupled with each zygote's genetics and epigenetic imprints, trace a life history path of health outcomes. The central theme of the Pittsburgh Specialized Center of Research on Sex and Gender Factors Affecting Women's Health is the "Genetic And Environmental Causes Of Adverse Pregnancy Outcomes." This major, but still under investigated, priority for women's health urgently requires multidisciplinary research both for the health of adult women and also for the health of developing fetuses and infants. For women, recurrent spontaneous abortions (RSA) are devastating. We have identified transgenerational transmission of a 'miscarriage gene' that may be an extreme example of deviant genomic imprinting. The implications for fetal outcomes are also of great importance, since in utero development of the fetus, both female and male, establishes the very foundation of the infant, adolescent, and adult. Three research projects along with two research cores and an administrative core are proposed under the directorship of Gerald Schatten, Ph.D. and Sarah Berga, M.D., Clinical Director. Project I, "Pregnancy Loss: Genomic Imprinting and Skewed X-Inactivation" (J. Richard Chaillet, MD, Ph.D., P.1), investigates DNA methylation defects in mice responsible for genomic imprinting as well as skewed X-chromosome inactivation, responsible for RSA in women, Project 11, "Epigenetic, Genetic and Environmental Regulation of Pregnancy in Primates" (Gerald Schatten, Ph.D., P.I. and Steve Caritis, MD, Co-P.I.), imaging primate pregnancies and inflammatory responses, addresses sex-specific genomic imprints in genetically controlled and experimentally-manipulated pregnancies. Project III (Julie DeLoia, Ph.D., P.I.), "Maternal and Fetal Consequences of Tobacco Smoke Exposure", analyzes the consequences of smoke exposure in pregnant women and in murine models to understand the interaction of 'genetic variants that jeopardize fetal development and pregnancy. The Imaging Core A performs noninvasive micro-PET and MRI imaging with specific probes, including transgenic MR11PET reporters. The Pregnancy Core B establishes and maintains pregnancies through conventional and artificial reproductive technologies (ART) in non-human primates and mice. The Administrative Core fosters intra- and inter-SCOR cooperation to facilitate and accelerate basic and clinical research. The multi-disciplinary, interactive, and collegial environments the new Pittsburgh Development Center at Magee-Women's Research Institute right on the contiguous campuses of the University of Pittsburgh and Carnegie-Mellon University, and brings together accomplished teams of clinical and basic investigators inspiring innovations in non-invasive imaging of pregnancy outcomes. Taken together, this comprehensive investigation will answer major women's health problems regarding the dynamic interplay among fetal and maternal genetics, sex-specific genomic imprints and consequences of our first environmental exposures. As such, it is an appropriate and complementary contributor to the ORWH's new SCOR program.

Title: Pre & Postnatal Cigarette Exposure and Infant Regulation
Principal Investigator: Schuetze, Pamela
Institution: Buffalo State College, Buffalo, NY
Funding Agency: National Institute of Child Health and Human Development
Project ID: HD039645
Project Funding Period: 1 September 2002 – 30 June 2004

Abstract: The purpose of this study is to investigate the impact of pre- and postnatal exposure to cigarettes and associated risk factors on infant regulation. Regulation during infancy is defined by the ability to modulate autonomic processes by maintaining physiological homeostasis as well as the ability to modulate responsiveness to both nonsocial and social stimuli. Difficulties with these regulatory processes may interfere with attentional processes and the infant's ability to successfully cope with sensory challenges from the external environment. While several studies have indicated that prenatal exposure to cigarettes is associated with altered regulatory functioning in neonates, it is unclear whether these effects persist beyond the neonatal period. Furthermore, the impact that postnatal exposure to cigarette smoke has on regulatory processes has received almost no attention. This study will examine the possibility that early exposure to cigarettes may impact regulation beyond the neonatal period through several pathways. The first is the direct teratological impact of prenatal cigarette exposure on regulatory processes. The second potential pathway is the impact of exposure to environmental cigarette smoke (passive smoking) on regulatory processes. A third pathway may be through the impact of maternal cigarette use on growth outcomes that, in turn, influence infant reactivity and regulation. The protocol consists of examining mother-infant dyads at 2 weeks of age and again at 7 months of infant age. The final sample will consist of 60 infants who were prenatally exposed to cigarettes, 60 infants who were passively exposed to cigarette smoke and 60 infants who were not exposed to cigarettes either prenatally or postnatally. Assessments of physiological (vagal tone) and behavioral reactivity and regulation will be conducted at both assessment points. In addition, mother-infant interactions and the quality of the caregiving environment (quality of home environment and maternal psychopathology) will be assessed. The study will provide information about potential teratogenic effects of cigarette exposure on regulatory processes. It will also allow a preliminary examination of potential pathways to dysregulation. Such knowledge may have significant implications for prevention programs designed to ameliorate regulatory disturbances among children exposed to cigarettes.

Title: Transplacental Pancreatic Carcinogenesis by NNK
Principal Investigator: Schuller, Hildegard
Institution: University of Tennessee, Knoxville, TN
Funding Agency: National Cancer Institute
Project ID: CA42829
Project Funding Period: 1 January 1998 – 31 July 2001

Abstract: This is a revised competing continuation proposal to study transplacental pancreatic carcinogenesis induced by coadministration of ethanol and the nitrosamine carcinogen NNK in a hamster model. Pregnant hamsters are administered 10% ethanol in drinking water from day 5 through day 15 of gestation and are then given a single intratracheal dose of 50 mg/kg NNK on day 15. The offspring develop adenocarcinomas of the exocrine pancreas with 50% incidence (males) and 77% incidence (females) as well as pancreatitis and marked acinar and ductular cell hyperplasia. Dr. Schuller proposes to investigate the mechanisms of carcinogenesis in this model. The general hypothesis to be tested appears to be that through a combination of b-adrenergic actions and in situ bioactivation to reactive intermediates, NNK initiates pancreatic tumorigenesis. The specific aims are 1) to investigate the role of the b-adrenergic receptor pathway in the initiation and development of pancreatic tumors; 2) to investigate the modulation of NNK metabolism and DNA adduction in fetal liver and pancreas by ethanol, 3) to identify

mutations induced in K-ras and p53 genes in hamster pancreatic tumors; 4) to investigate the roles of individual cytochrome P450 enzymes in the bioactivation of NNK in fetal pancreas in vitro and in vivo; and 5) to investigate the ability of the nonsteroidal antiinflammatory agent sulindac to modulate pancreatic carcinogenesis. It is proposed that these studies will serve as the basis for developing effective strategies for prevention of cancer in the children of smokers.

Title: Adenocarinoma of the Lung in Women
Principal Investigator: Schwartz, Ann G.
Institution: Wayne State University, Detroit, MI
Funding Agency: National Cancer Institute
Project ID: CA087895
Project Funding Period: 13 June 2001 – 31 May 2006

Abstract: In 1998, 80,000 women in the US were diagnosed with lung cancer and incidence rates, particularly of adenocarcinoma, continue to increase among women. Many pieces of evidence suggest that there are gender differences in susceptibility to tobacco carcinogens. Several studies have shown that DNA adducts, p53 mutations, CYP1A1 expression in the lung, and GSTM1 null genotypes are more frequent in females than in males. Reasons for differential susceptibility by gender might be explained by variations in metabolic enzyme functioning or hormonal differences. Some of the same enzymes involved in the metabolism of carcinogens in tobacco smoke are involved in the metabolism of estrogen. The goals of the proposed study are two-fold. First, we will evaluate the role of tobacco smoke and estrogens in determining risk of adenocarcinoma of the lung among women. Secondly, we will evaluate the role of estrogen receptors and c-erbB-2 in lung tumors to further understand the pathways through which estrogen may be acting in the lung. The specific aims are: 1) To conduct a population-based case-control study of the contribution of tobacco exposure, estrogen use, and reproductive history in determining risk of adenocarcinoma of the lung in women. 716 cases will be identified through the Metropolitan Detroit Cancer Surveillance System of the Karmanos Cancer Institute (a SEER participant). An equal number of controls will be selected through random digit dialing. 2) To determine if genotype at the metabolic enzyme loci CYP1A1, CYP1B1, CYP17, CYP19, GSTM1, GSTP1, COMT, and NQO1 are associated with risk of adenocarcinoma of the lung in women. These enzymes are active in both the metabolism of tobacco smoke carcinogens and the synthesis and metabolism of estrogens. 3) To examine gene-gene and gene-environment interactions, focusing on tobacco and estrogen effects. 4) To determine estrogen receptor status (alpha and beta) and c-erbB-2 levels in the lung tumors of women with adenocarcinoma and evaluate risk associated with tobacco exposure, estrogen use, reproductive history, and genotype at metabolic enzyme loci by tumor characteristics. The proposed study represents a focused approach to defining the contribution of genes and environments in risk of adenocarcinoma of the lung in women. The interview component of the study will provide data about individually measured environmental risk factors. Genotypes have been chosen which impact on biologically effective dose of tobacco carcinogens and estrogens in the lung. The study of tumor characteristics will provide insight into mechanism of action. This large, population-based study should provide clues for important prevention and therapeutic strategies for lung cancer.

Title: Fetal & Adolescent Nicotine Effects on CNS 5HT Systems
Principal Investigator: Slotkin, Theodore A.
Institution: Duke University, Durham, NC
Funding Agency: National Institute on Drug Abuse
Project ID: DA014247
Project Funding Period: 1 September 2001 – 31 May 2004

Abstract: We have developed rodent models of prenatal nicotine exposure that simulate the plasma levels found in smokers or in users of the transdermal nicotine patch, and have demonstrated that nicotine itself is a neuroteratogen that elicits synaptic functional changes appearing after an extended period of apparent normality. These effects target catecholamine systems and we have preliminary data indicating involvement of 5HT systems as well. Brain development continues into adolescence, the period in which nearly all smokers begin smoking, and we have developed a comparable rodent model of adolescent nicotine administration; again, catecholamine systems are targeted and we have preliminary data for 5HT. It is clear that a subset of smokers are using tobacco to self-medicate for depression; additionally, adolescent smoking is associated with higher incidence of subsequent depression. These findings lead to the current hypothesis: namely that nicotine, during a critical period of brain development, alters the set-point for 5HT activity, at the level of presynaptic function, at receptor signal transduction cascades, or both. This will be pursued in fetal and adolescent nicotine exposure models, utilizing neurochemical, cell signaling, and behavioral approaches. Aim 1. To determine how prenatal nicotine exposure alters 5HT synaptic function and behaviors known to be targeted in models of 5HT dysfunction. Evaluate development of 5HT projections, using nerve terminal markers, 5HT turnover, and the ability of acute nicotine challenge to release 5HT; studies conducted from birth to adulthood. 5HT signal transduction assessed with receptor ligand binding and linkages to adenylyl cyclase. Aim 2. To determine whether the critical period for nicotine- induced alterations in the programming of 5HT function extends into adolescence. We will assess 5HT synaptic function and behaviors during adolescent nicotine treatment and withdrawal, using the same endpoints as studied with the prenatal nicotine model. Aim 3. To determine whether prenatal nicotine exposure alters the response to subsequent adolescent nicotine administration. Animals exposed prenatally to nicotine will receive adolescent nicotine treatment and the response of 5HT systems and behavior will be assessed, along with catecholaminergic responses and nicotinic receptors known to be affected by adolescent nicotine.

Title: Effects of Prenatal Exposure to Nicotine on Primate Lung Development
Principal Investigator: Spindel, Eliot R.
Institution: Oregon Health & Science University, Portland, OR
Funding Agency: National Center for Research Resources
Project ID: RR000163-430016
Project Funding Period: Not available

Abstract: The deleterious effects of maternal smoking during pregnancy are well established. Maternal smoking is the major preventable cause of intrauterine growth retardation and prematurity. Perhaps less well appreciated, is the recent, strong evidence, that smoking during pregnancy directly and adversely affects lung development. Respiratory problems associated with in utero tobacco exposure include decreased lung function, increased respiratory diseases and increased incidence of sudden infant death syndrome (SIDS). Given the unfortunate prevalence of smoking during pregnancy and the resulting serious consequences, it is of major importance to understand the mechanisms underlying smoking-induced changes in the newborn. We have begun to investigate this by administration of nicotine to timed-pregnant rhesus monkeys. In preliminary studies we have demonstrated that exposure of pregnant rhesus monkeys to a nicotine dose consistent with that of smokers alters fetal airway development and that related effects can be reproduced in fetal monkey lung organ culture.

Immunohistochemistry shows wide expression of nicotinic receptors in developing lung and nicotine appears to alter the pattern of receptor expression. Preliminary data further suggests that some of the effects of nicotine, acting though nicotinic receptors, may be mediated by antagonism of the mitogenic effects of peptide growth factors such as GRP. From these studies will come the first description of the effects of chronic nicotine exposure on lung function, a determination of the extent to which these effects are reversible, and a beginning understanding of the mechanisms underlying these effects.

Title: Fetal Nicotine Exposure Effect on Primate Lung
Principal Investigator: Spindel, Eliot R.
Institution: Oregon Health & Science University, Portland, OR
Funding Agency: National Institute of Child Health and Human Development
Project ID: HD037131
Project Funding Period: 1 February 1999 – 31 January 2004

Abstract: The deleterious effects of maternal smoking during pregnancy are all too well established. Maternal smoking is the major preventable cause of intrauterine growth retardation and prematurity. Perhaps less well appreciated, is the recent, overwhelming evidence, that smoking during pregnancy directly and adversely effects lung development. Respiratory problems associated with in utero tobacco exposure include decreased lung function, increased respiratory diseases and increased incidence of sudden infant death syndrome (SIDS). Given the unfortunate prevalence of smoking during pregnancy and the resulting serious consequences, it is of major importance to understand the mechanisms underlying smoking-induced changes in the newborn. Our preliminary data suggests that nicotine itself is one of the factors responsible for the changes in pulmonary function observed in neonates born to smoking mothers. In this application we propose to use the rhesus monkey to characterize the effects of chronic exposure to low levels of nicotine throughout pregnancy on lung development and subsequent pulmonary function. Whole animal studies will be complemented with in vitro studies to begin to determine the molecular mechanisms underlying nicotine's effect on lung. In preliminary studies we have demonstrated that exposure of pregnant rhesus monkeys to a nicotine dose consistent with that of smokers alters fetal airway development and that related effects can be produced in fetal monkey lung organ culture. Immunohistochemistry shows wide expression of nicotinic receptors in developing lung and nicotine appears to alter the pattern of receptor expression. Preliminary data further suggests that some of the effects of nicotine, acting through nicotinic receptors, may be mediated by antagonism of the mitogenic effects of peptide growth factors. Thus we specifically propose to 1, Determine the basis for nicotine's actions by determining the time course and cell specific expression of nicotinic receptor subtype expression in fetal monkey lung; 2, Characterize the effect of fetal exposure to nicotine on lung development and function by functional, morphometric, immunohistochemical and molecular analysis; and 3, begin to determine the mechanism underlying nicotine's actions by use of fetal monkey lung organ culture. From these studies will come the first description of the effects of chronic nicotine exposure on lung function; a determination of the extent to which these effects are reversible; and a beginning understanding of the mechanisms underlying these effects. Definitive knowledge of the effects of nicotine on lung development would provide an important additional tool in smoking control and will begin to better explain the link between maternal smoking and altered neonatal respiratory function.

Title: Fetal Neurodevelopment—Effects of Nicotine and Hypoxia
Principal Investigator: Stark, Raymond I.
Institution: Columbia University Health Sciences, New York, NY
Funding Agency: National Institute of Child Health and Human Development
Project ID: HD013063-230015
Project Funding Period: Not available

Abstract: Prenatal exposure to nicotine through maternal smoking leads to alterations in fetal responses related to arousal and cardiorespiratory control far beyond the period of exposure. The drug puts fetuses at increased risk for growth restriction, prematurity, perinatal complications and after birth, for the Sudden Infant Death Syndrome, behavioral and learning disabilities, and attention deficit disorders; while the adolescent female offspring may be at increased risk for becoming smokers themselves. By hypoxia and direct effects of nicotine on fetal neurodevelopment have been implicated as mechanisms for this array of consequences. Our overall hypothesis is that excessive and untimely stimulation of fetal nicotinic receptors by nicotine induces wide ranging structural and functional changes in the developing nervous system leading to alterations in central regulatory mechanisms controlling autonomic and behavioral functions. While fetal regulatory mechanisms adapt to chronic nicotine exposure to maintain a relatively "normal" physiology, hypoxic stress will reveal deficiencies in physiologic competence. These structural functional alterations, established in utero produce a "vulnerable" newborn who will have a life long risk for stress related pathologies. To test these hypotheses, we will compare key markers of neurophysiologic function (coordinated fetal states, response to hypoxia and baroreceptor gain) in nicotine exposed fetuses with controls and relate their functional impairments to structural differences in brainstem and forebrain arousal and cardiorespiratory centers. Studies are carried out in a unique chronically instrumented baboon model. The homologies in neurodevelopment between the human and baboon fetus make knowledge gained from this research relevant to identifying high risk fetuses and infants.

Title: Response to Hypoxia and Nutrition During Development
Principal Investigator: Stark, Raymond I.
Institution: Columbia University Health Sciences, New York, NY
Funding Agency: National Institute of Child Health and Human Development
Project ID: HD013063
Project Funding Period: 1 August 1979 – 30 June 2005

Abstract: The central theme of this Program is based on the axiom that, during early stages of development, organisms are uniquely vulnerable to environmental challenges that constrain the physiological and behavioral phenotypes that are manifest throughout the rest of the life span of the organism. Understanding the mechanisms that confer risk or resistance to these challenges is the fundamental goal of the work done in all of the projects. The first Project, "Activity and Responses to Hypoxia/Nicotine in Development" examines the risks conferred on the developing fetal baboon by nicotine in smoking cessation programs for pregnant women and in understanding postnatal vulnerabilities of these infants for SIDS, attention deficits, and other neurobehavioral disorders. The second project, "Activity and Responses to Nutrient and Oxygen Supply" focuses on nutritional challenges experienced by the growth restricted fetal lambs and by very low birth weight infants during their adjustment to extra- uterine life. Knowledge of the short-term physiological responses and adaptations to variation in nutrient supply are the logical starting point for understand the long-term risks associated with inadequate nutrition early in life. The third project, "Perinatal Nutrition and Mechanisms of Adult Disease", addresses the long-term consequences of nutrient deprivation early in life. Research focuses on changes in placental and fetal gene expression that represent proximal steps associated with nutrient programming, the role of endogenous versus exogenous factors in stabilizing the programmed phenotype and

how later nutrition and growth serve to amplify effects of fetal programming. An administrative, statistical and computer Core and molecular/bioanalytical Core support the needs of the research.

Title: Fertility, Smoking and Early Mammalian Development
Principal Investigator: Talbot, Prudence
Institution: University of California, Riverside, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 6RT-0039
Project Funding Period: 1 July 1997 – 30 June 2001 (and 10RT-0239 renewal until 30 June 2004)

Abstract: Human reproductive health is affected by smoking. We propose to characterize the effects of tobacco smoke on reproduction, identify the toxicants in tobacco smoke that affect reproduction, understand the mechanism of action of these toxicants, and develop methods to control or prevent their effects. Specifically, active smoking increases the risk of infertility, spontaneous abortion, and ectopic pregnancy. These reproductive disorders/diseases have increased in the past several decades. In the United States, as many as 1 in 6 couples are infertile. Ectopic pregnancy, which is the leading cause of maternal death during the first trimester and which is almost always fatal to the fetus, has quadrupled in the United States since 1970. Our previous work has shown that both the oviduct and corpus luteum of females are targets of mainstream (MS) and sidestream (SS) cigarette smoke. The oviduct and corpus luteum are important reproductive organs; impairment of their function by smoke could cause infertility, spontaneous abortion, and ectopic pregnancy. We propose to characterize the effects of MS and SS smoke on the oviduct and the corpus luteum, determine if the effects can be reversed, identify the toxicants in smoke that produce these effects, and begin to clarify the mechanism of action of the toxicants. Most work will be done using the hamster as a model, some parallel experiments will be done on human oviducts, and some experiments will be done on cultured human endothelial cells Oviducts. To characterize the structural and functional effects of MS and SS on the living oviduct, hamsters will be placed in smoking machine experiments, and the target cells in their oviducts will be evaluated using electron microscopy and physiological tests. Any para-meter that is affected by smoke exposure will then be examined in a reversal experiment to determine if "giving up" smoking restores the normal condition. Finally, human oviducts from nonsmokers, active smokers, and passive smokers undergoing elective surgery will be studied to characterize the structural and functional effects of smoke on the human oviduct Corpus luteum. Our previous work shows that MS and SS smoke inhibit blood vessel development in corpora lutea. Our second goal is to study this effect in more detail. We will characterize the structural effects of smoke on the hamster corpus luteum and on a chick membrane used to assay blood vessel development. Finally, we will determine how the genes which regulate blood vessel development are affected by smoke. The genes which are affected by the toxicant will be sequenced and their expression will be studied in the corpora lutea of hamsters in smoking machine experiments. Results from these studies may influence smoking behavior in young women of child-bearing age. Young people are more likely to stop smoking if they know its effects are imminent (childbearing) rather than remote (death). Our video tapes showing the rapid response of the reproductive organs to smoke inhalation will be made available for educational purposes and may deter young women from smoking. Our video laparoscopy technique may become widely established for toxicology testing; this technique has the potential to be applied to other organs, routes of delivery, toxicants, and to males. The toxicants in smoke, once identified, could be removed form cigarette smoke by appropriate filters, thereby decreasing the toxicity of smoke to women unable to stop smoking during their reproductive years. Passive smokers are often unaware of the dangers of smoke exposure. Our experimental designs compare MS and SS smoke and thus will provide much needed data on the influence of passive smoking on reproductive events. Finally, our studies will benefit other branches of medicine. Results on the oviduct may apply to other organs having ciliated cells and

smooth muscle cells, such as the respiratory system, and studies on the corpus luteum will contribute to understanding blood vessel development at other sites, such as in wounds, the endometrium, the placenta, and the embryo/fetus. In addition our studies on blood vessel development may augment our understanding of diseases affected by blood vessels such as solid tumor growth, and rheumatoid arthritis. In summary, data obtained in this study will lead to health improvements for pregnant women and their fetuses, and will have a positive impact on the reproductive health of our species.

Title: Smoking During Pregnancy: Chromosome Damage in Mothers and Newborns
Principal Investigator: Tucker, James
Institution: Lawrence Livermore National Laboratory, Livermore, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 8RT-0070H
Project Funding Period: 1 July 1999 – 30 June 2002

Abstract: The consequences of maternal cigarette smoking during pregnancy on both the mother and newborn are being studied. It is of particular interest to understand how smoking and inherent genetic susceptibility relate to observed chromosomal aberrations in circulating blood cells. Approximately one quarter of the population is inherently susceptible to chromosomal damage whereas the remaining three quarters are relatively resistant. It is hypothesized that sensitive populations of newborns and mothers are at increased risk to chromosomal damage by maternal smoking during pregnancy compared to relatively resistant populations.

Blood samples from 470 mothers and their newborns have been collected for this study. Peripheral blood lymphocytes from the mother and the fetal side of the placenta were cultured and harvested 48 and 72 hours later to evaluate chromosomal aberrations and genetic susceptibility, respectively. Chromosome aberrations are detected using whole chromosome painting probes to visualize chromosomes 1, 2, and 4 in red and 3, 5 and 6 in green. Approximately 1000 cell equivalents (1800 metaphase cells) are being scored from the maternal and newborn samples to identify stable and unstable chromosome damage. The clastogen, bleomycin, is used to assess susceptibility to induced chromosomal damage in vitro.

In addition to baseline and postpartum interview questionnaires data about smoking history, some maternal and newborn blood samples are being tested by our collaborators for two biochemical measures of tobacco exposure. The quantification of cotinine levels and 4-aminobiphenyl-hemoglobin (4-ABP-Hb) adducts will reduce the risk of potential recall bias for self-reported tobacco use. Preliminary data indicate that self-reported cigarette smoking behavior prior to and early in pregnancy are highly correlated with these biochemical measures of exposure.

To date, 202 mother/newborn blood sample pairs have been analyzed for chromosomal damage: 106 are from Caucasian-Americans and 96 are from African-Americans. Preliminary statistical analyses have been performed on a subset of these subjects, namely 97 Caucasian American and 73 African American sample pairs. The distribution of bleomycin-induced chromatid damage in both the maternal and newborn populations deviates from a normal distribution. In general, the newborn lymphocytes are more resistant to bleomycin-induced damage than the maternal cells. The most recent analyses of the data evaluated the effect of age, race, maternal smoking during pregnancy, ever smoking, passive smoking, and bleomycin sensitivity on chromosome aberration frequency. In univariate analyses, there are significant associations between maternal chromosome aberration frequencies and mother's age ($p = 0.05$) and passive smoke exposure ($p = 0.01$) and these factors remain significant in multivariate analysis. In univariate analyses of data for newborn samples, bleomycin sensitivity associates significantly with chromosome damage.

In a multivariate analysis, ever smoking, smoking during pregnancy, and passive smoke exposure are significantly associated with chromosome damage.

We have now finished collecting the samples. In the next few months we will finish the cytogenetic analyses. We will determine if maternal and newborn populations, genetically susceptible to DNA damage, are at increased risk of chromosomal aberrations due to tobacco exposure during pregnancy. The identification of risks associated with maternal smoking during pregnancy are critical for the improvement of the health of the individual and the community.

Title: Effects of Passive Smoking and Pregnancy
Principal Investigator: Turteltaub, Kenneth
Institution: Lawrence Livermore National Laboratory, Livermore, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 8RT-0072
Project Funding Period: 1 July 1999 – 30 June 2002

Abstract: Studies in people have suggested that second-hand smoke from cigarettes can cause cancer in people who never smoked. Importantly, some of the most vulnerable non-smokers to second-hand cigarette smoke may be to newborn children. These studies, while implicating second-hand smoke as a human health threat, do not provide information on how this process occurs. Information on the way second-hand smoke causes cancer will aid significantly in our understanding of the risk this poses to the public and to children.

Smoking-related cancers are believed to involve chemicals present in the smoke. Passive smoke, which is emitted from the burning end of the cigarette, contains higher concentrations of these chemicals than main stream smoke. Upon inhalation, these chemicals pass from the smoke into the bloodstream and chemically bind to proteins and the genetic material of cells, DNA, forming adducts. DNA and protein adducts have been detected in the sperm of adult men who smoke and in the placenta and umbilical cords of newborn children of women who smoke. However, there has been no information to show that second-hand smoke causes this damage. There is also no direct method to measure these adducts at levels of chemical exposure equivalent to second-hand smoke.
The purpose of this work will be to specifically determine if DNA and protein adducts are formed when exposed to two chemicals present in cigarette smoke, nicotine and hydroquinone, at levels present in side-stream smoke. These chemicals are important components of the smoke. This will be possible using the novel and sensitive technique of accelerator mass spectrometry. This research will determine whether adduct levels increase with increasing dose and will help identify whether the adducts of these common components of cigarette smoke can be used to measure side-stream smoke exposure. This research will also help determine if this damage occurs in the offspring of smoking mothers.

Title: Effect of Smoke and Gender on Bronchiolar Injury and Repair
Principal Investigator: Van Winkle, Laura
Institution: University of California Davis, Davis, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 11RT-0258
Project Funding Period: 1 July 2002 – 30 June 2005

Abstract: Environmental tobacco smoke (ETS) is a substantial public health hazard because of the large number of smokers and the widespread presence of ETS that can lead to involuntary exposure of nonsmokers. ETS exposure is causally associated with lung cancer in both adult men and women. However, important differences exist in susceptibility to lung cancer between

men and women. The risk for all major lung cancer types is higher in women than in men at every level of exposure to tobacco smoke. Recent research has shown that women express certain genes at increased levels in response to tobacco smoke. These changes are not seen in men exposed to tobacco smoke. While these changes suggest that there are genetic reasons for the differences in lung cancer risk between men and women, there have been few studies that define the biologic basis for these differences.

The proposed research will focus on the cellular changes that occur in mice exposed to tobacco smoke and will determine if these changes are different in female mice compared to male mice. Subtle effects such as cellular changes in response to ETS in males and females have not been thoroughly investigated. Furthermore, because these subtle changes may be very focal within the lung the experimental approach must use methods that are also site-specific and that can evaluate cellular changes in focal lung regions. Our laboratory frequently uses and develops new site-specific methods such as these and will apply them in the proposed studies. Preliminary data from our laboratory suggest that female mice are more sensitive to an air pollutant, naphthalene, that is found in both cigarette smoke and car exhaust. We feel that coexposure to tobacco smoke and excess naphthalene in air pollution may result in an accentuated effect (such as cell changes that lead to cancer or chronic bronchitis) in female animals. Our hypothesis in the proposed studies is that female mice will have increased lung injury compared to males. We will try to determine the mechanism for the cellular changes.

This research has potential impact for women who smoke or are exposed to ETS and who live in urban areas with high levels of air pollution. We expect these studies will benefit the general population by defining the mechanisms that make women more susceptible. Once this mechanism has been defined strategies can be devised that will afford sensitive populations some protection. These studies are highly relevant to TRDRP research priorities regarding tobacco smoke exposure in a sensitive population, women.

Title: Retinoids and Nicotine: Effects on Alveolar Development
Principal Investigator: Veness-Meehan, Kathleen A.
Institution: University of British Columbia, Vancouver, BC
Funding Agency: National Heart, Lung, and Blood Institute
Project ID: HL068377
Project Funding Period: Not available

Abstract: Normal lung development is a complex process that may be altered by exposures during critical periods. Nicotine and nicotine containing products are used by an estimated 400,000 pregnant women annually in the U.S. and studies indicate that exposure of the developing lung to nicotine impairs alveolar development and may result in long term decreases in pulmonary function. Retinoic acid has been shown to promote alveolar development, attenuate glucocorticoid-induced inhibition of alveolarization in rats and induce septal formation in mice with a genetic failure of lung septation. We hypothesize that retinoic acid treatment will promote alveolar development in rats exposed to nicotine *in utero* and during the first 2 weeks of life. To test this hypothesis, rats will be treated with nicotine during pregnancy and for 14 days postpartum and their offspring will be treated with retinoic acid during the period of alveolar formation. Changes in lung structure, cell proliferation, apoptosis, differentiation and ECM deposition will be assessed by quantitative morphology, immunohistochemistry and TUNEL staining.

Title: Prenatal Smoking and Preschool Behavior Problems
Principal Investigator: Wakschlag, Lauren S.
Institution: University of Chicago, Chicago, IL
Funding Agency: National Institute on Drug Abuse
Project ID: DA000330
Project Funding Period: 30 September 1997 – 31 August 2002

Abstract: Research establishing links between mothers' smoking during pregnancy and children's behavior problems suggests a significant, potentially modifiable contributor to one of the most serious mental health disorders of childhood. To establish the etiologic significance of prenatal smoking for specific types of disruptive behavior disorders, however, further prospective research is needed. The proposed project aims to provide advanced training/mentorship to the candidate in the conduct of research examining the relation of prenatal exposure to cigarette smoke and young children's behavior problems. The candidate is a clinical-developmental psychologist who seeks this training towards her goal of studying the effects of prenatal substance exposure on the development of behavior problems. Her existing expertise in the study of parent-child relationships and the clinical assessment of young children will be supplemented by: a) ongoing mentorship in regard to design and implementation of longitudinal research examining effects of prenatal substance exposure; b) advanced training in biochemical measurement and neuropharmacological effects of smoking, and longitudinal data analysis and; c) conduct of independent research. The proposed research is a prospective examination of prenatal smoking and behavior problems in high-risk preschoolers, designed to identify pathways by which smoking may increase the risk of behavioral symptomatology. Participants in an ongoing study of low-income African-American families will be recruited for this preschool follow-up assessment. Prospective data include repeated measures of pre- and postnatal smoking, parenting and infant development. 105 mother-child pairs will be seen including prenatally, actively passively, and non-exposed children. Multi-method assessment of behavior problems will include parent/examiner ratings, structured psychiatric interviewing, laboratory assessment and behavioral ratings during parent-child interaction. Child developmental functioning, parental psychopathology and parenting practices will also be assessed. Multivariate planned comparisons will be used to examine the relation of pre-and postnatal exposure to smoke and preschool behavior problems. Structural equation modeling will be used to identify direct and indirect effects of smoking with particular emphasis on causal pathways between smoking, parental psychopathology and the quality of the parent-child relationship.

Title: Prenatal Smoking and Aggression in Twins
Principal Investigator: Ward, Michelle C.
Institution: University of Southern California, Los Angeles, CA
Funding Agency: National Institute on Drug Abuse
Project ID: DA006076
Project Funding Period: Not available

Abstract: The proposed study aims to understand the relationship between prenatal smoke exposure and aggression in children. The study will expand on previous findings by also investigating the extent to which this relationship may be mediated by genetic factors; a mother's smoking behavior during pregnancy may be influenced by her own predisposition toward antisocial behavior. Aggression, along with other traits such as cognitive ability in 9 year old twins will be measured using in depth interviews and multiple informant behavior assessments. The mothers will be assessed for antisocial behavior in similar manner, including ascertainment of current and prenatal substance abuse. This study will be conducted in a genetically informative twin design, which will allow for the investigation of the smoking/aggression relationship while controlling for covariates. It is hypothesized that children exposed in utero to high levels of cigarette smoke will exhibit elevated aggression. Also hypothesized, however, is

that the said relationship will be at least partially explained by other risk factors, such as genetic predisposition towards anti-social behavior in the mother. Birth complications will also be examined for possible exacerbation of the smoking/aggression relationship as evidenced in prior studies. The effects of smoke exposure are expected to be greatest in the presence of birth complications.

Title: Premenopausal Hormone Levels and Risk of Breast Cancer
Principal Investigator: Willett, Walter C.
Institution: Harvard University School of Public Health, Boston, MA
Funding Agency: National Cancer Institute
Project ID: CA067262
Project Funding Period: 10 May 1996 – 28 February 2002

Abstract: The epidemiology of breast cancer suggests a central etiologic role for premenopausal endogenous sex hormones, yet current data do not indicate which specific endogenous hormones (or hormone fractions) are of greatest importance and at what levels risk is increased Similarly, current data suggest that urinary estrogen metabolites and plasma antioxidants may also be related to breast cancer risk yet these relations have not been assessed in a large prospective study The Nurses' Health Study Il (NHSII) is a large ongoing prospective cohort study of 116,678 female, U.S. registered nurses who were 25-42 years of age when the study began in 1989. We now propose to collect and archive blood and urine samples from approximately 40,000 premenopausal women participating in the NHSII who are at least 35 years of age, are free from cancer, have completed previous NHSII questionnaires, are not using exogenous hormones and are neither pregnant nor breastfeeding. Plasma drawn during both the follicular phase (days 3-5) and luteal phase (approx days 20-22), white and red blood cells, and a urine sample will be stored in liquid nitrogen freezers and later analyzed using a nested case-control design. We propose to assess the following specific hypotheses (1) higher levels of estrogens, androgens, prolactin and progesterone in both the follicular and luteal phase of the menstrual cycle each increase risk of breast cancer, (2) higher levels of plasma beta-carotene and other carotenoids, retinol, and alpha-tocopherol reduce the risk of breast cancer and (3) the ratio of 16-alpha to 2-hydroxylated urinary metabolites is positively associated with an increased risk of breast cancer. The ongoing NHSII will provide follow-up of the cohort and documentation of breast cancer (CA 50385) in addition to information on important covariates (such as body mass index, parity, age at menarche, smoking status and dietary intake, among others) for the proposed study. Overall, the large size of the cohort, the prospective design, the high follow-up rate, the detailed covariate data, and the ability to collect blood specimens timed according to the menstrual cycle provide a unique opportunity to evaluate several important hypotheses related to breast cancer risk.

Title: Effects of Tobacco Smoke Exposure on Hormones and Fertility
Principal Investigator: Windham, Gayle
Institution: Sequoia Foundation
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 7RT-0119
Project Funding Period: 1 July 1998 – 29 June 2001

Abstract: Cigarette smoke is known to contain a large number of compounds, some of which are suspected to cause damage to the reproductive system. Smoking is one of the most preventable causes of low birth weight in exposed babies and has also been associated with pregnancy loss, infant death and infertility. Some evidence also suggests that smoking may affect hormone levels, which in turn could affect pregnancy and other hormone-dependent conditions. For example, women who smoke tend to reach menopause earlier than non-smokers.

We previously conducted a study that looked at different aspects of the menstrual cycle. Women kept track of their periods and collected urine daily during several cycles. From the urine , the levels of two hormones which are secreted from the ovaries during each "normal" menstrual cycle, namely estrogen and progesterone, were measured. We found that menstrual cycles of heavy smokers tended to be shorter and more irregular than those of non-smokers. There also appeared to be some differences in hormone patterns by smoking status. Shorter cycles (in general) have been linked to earlier menopause and to higher risk of breast cancer. The timing of events in the menstrual cycle, including release of progesterone and estrogen, may affect the ability to get and stay pregnant, so these findings are important for reproductive fitness. This is one of the first studies to look at smoking and menstrual function in this way and the exact means by which smoke exposure may cause these changes is not clear. Therefore, we plan to pursue these findings further in this valuable database.

The release of hormones by the ovary is under the control of messages from other organs, such as the pituitary and the brain. To learn more about how smoke exposure may act on this system, we will measure one of the pituitary hormones, called follicle stimulating hormone (FSH). FSH is important in preparing for ovulation (release of the egg in the middle of the cycle) and thus may be a marker of ovarian function. Two prior studies found some increase in FSH among smokers, but FSH was measured on only a few days. We will measure FSH on each day of a sample of menstrual cycles, so we can examine patterns throughout the cycle. FSH production will be compared by smoking status and to other menstrual cycle characteristics to determine whether it is involved in the changes in menstrual function we noted among smokers in this study.

This study is directly relevant to the goals of the TRDRP to understand the biomedical effects of smoking. It is cost-effective because it uses information from a database of over 400 women and 2,000 menstrual cycles which was already collected at considerable cost and time. From this study, we expect to learn more about how smoking affects hormone systems and reproductive function. These hormone system, in turn, are critical for many other aspects of women's health.

Title: Nicotine and Nicotinic Signaling Lung Development
Principal Investigator: Wuenschell, Carol W.
Institution: University of Southern California, Los Angeles, CA
Funding Agency: National Heart, Lung, and Blood Institute
Project ID: HL003786
Project Funding Period: 5 July 1998 – 30 June 2003

Abstract: The candidate's goal is to pursue an independent research career in the field of lung developmental biology. The candidate was trained as a developmental biologist at UCLA. Her graduate and postdoctoral training involved the use of a variety of molecular and genetic approaches to the study of gene expression in developing neurobiological systems. Her entry into the area of lung biology is relatively recent and represents a new direction for her career. The candidate holds a faculty appointment at the Center for Craniofacial Molecular Biology of the University of Southern California. This highly interactive, state-of-the-art research center has provided full support for the candidate's independent research efforts for the past two and a half years and is committed to her continued pursuit of a research career. The candidate plans to develop her career by acquiring new research skills and increasing her knowledge of lung biology and lung diseases through course work, and through formation of new collaborations and new contacts with clinicians and researchers at USC and elsewhere. The candidate is interested in understanding the mechanisms by which prenatal exposure to maternal smoking affects embryonic and fetal lung development, leading to the observed short and long term effects on the respiratory health of the offspring. Her Research Plan addresses the following hypothesis: Nicotine alters the developmental program of the embryonic lung (including expression of

surfactant protein genes) through a mechanism involving nicotinic acetylcholine receptors located on cells of the distal epithelium and stimulation of production of GRP by these same distal epithelial cells. The Specific aims are: 1) to characterize the effect of added nicotine on the development of embryonic mouse lungs grown in serumless culture with respect to specific gene expression, growth, branching morphogenesis and differentiation of epithelial cell types, 2) to determine the nature and location of nicotinic acetylcholine receptors mediating the developmental effects of nicotine in the lung and 3) to test the role of the peptide growth factor GRP and its receptor in mediating the effects of nicotine in embryonic lung.

Title: Nicotine/Neuroendocrine Signaling in Developing Lung
Principal Investigator: Wuenschell, Carol W.
Institution: University of Southern California, Los Angeles, CA
Funding Agency: National Heart, Lung, and Blood Institute
Project ID: HL054960
Project Funding Period: 1 April 1997 – 31 March 2003

Abstract: Prenatal exposure to maternal smoking adversely affects the respiratory health of children even in the absence of postnatal passive smoking. Transplacental nicotine exerts developments effects on fetal lung in animals, including lung hypoplasia and hyperplasia of pulmonary neuroendocrine cells. Paradoxically, the lungs of smoking-exposed infants appear to be more mature at birth than those of unexposed infants. The cellular and molecular mechanisms underlying these prenatal effects of maternal smoking are presently unknown. The study will use culture of whole embryonic mouse lungs in chemically defined medium as a model system to study the mechanism of nicotine action in development lung. Preliminary data show stimulation of branching and expression of the genes encoding the peptide growth factor GRP and surfactant proteins SP-C and SP-A in nicotine-treated lungs. The effect on SP-C gene expression is blocked by the nicotinic antagonist d-tubocurarine. Further, the increase in SP-C gene expression is blocked an antibody directed against the reactive region of GRP. Thus, the effects of nicotine in this system are mediated by nicotinic acetylcholine receptor (nAChR) subtype and GRP may be a mediator in the downstream pathway. The added observation that embryonic lung distal epithelial cells posses and partial neuroendocrine phenotype leads to this hypothesis; That nicotine alters the developmental program of the embryonic lung including expression of surfactant protein genes, through a mechanism involving nAChRs located on cells of the distal epithelium and stimulation of production of GRP by these name distal epithelial cells. The specific Aims are: 1) To characterize the effects of added nicotine on development of embryonic lung with respect to growth, branching morphogenesis, specific gene expression and differentiation of epithelial cells. 2) To determine the nature and location of the nAChRs mediating the developmental effects of nicotine in embryonic lung. 3) To test the role of GRP as a downstream mediator of nicotine effects by blocking strategies. The results of this study will provide insights into an aspect of normal lung development that has not previously been explored, as well as increasing our understanding of the mechanism of nicotine toxicity in developing lung and possible cancer risks from prenatal nicotine exposure.

Title: Smoking, Gender, Hormones and the Brain
Principal Investigator: Zaidel, Eran
Institution: University of California Los Angeles, Los Angeles, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 8IT-0112
Project Funding Period: 1 July 1999 – 30 June 2001

Abstract: Despite steady progress in the fight against nicotine addiction over the past 25 years, the percentage of female smokers is increasing. The United States may soon become the first

society in history in which more women than men smoke. In part, this is because women are less likely to be able to quit, whether they try on their own or with the help of smoking cessation programs. This appears to be especially true for nicotine replacement therapies (such as nicotine patches or gum), which have helped a greater percentage of men than women in almost every study. There is thus a clear need for new forms of treatment which are tailored especially for women.

Nicotine is an addictive drug, and the primary reason people use cigarettes is to get nicotine. However, other aspects such as the taste of smoke also become pleasurable to the smoker. These other aspects may be more important for women, and this could be one reason that nicotine in the patch and gum may be less likely to help them give up cigarettes. To test this theory, we will study the differences in men and women's reaction to nicotine alone, or to smoking a cigarette which contains very little nicotine.

Smokers often feel that cigarettes help them stay focused, and one of the symptoms of nicotine withdrawal is difficulty paying attention. In women, withdrawal symptoms and attentional ability may change at different points of the menstrual cycle. Our research will use a measure of attention to study the effect of smoking and withdrawal across the menstrual cycle. Our test is also able to determine differences between the right and left sides of the brain, and the effectiveness of the connections between the two sides. This is useful since gender, menstrual hormones, and withdrawal may be associated with changes in right/left balance. The brain controls smoking, as it does any other behavior. Knowledge of which brain areas are responsible will let new treatments to help smokers quit become more effective, by specifically targeting those areas. Our research will help provide this knowledge, with special emphasis on factors (attention, menstrual cycle, and right/left brain function) that are believed to be important to female smokers.

Title: Nicotine & Its Metabolites: Apoptosis in Developing Neurons
Principal Investigator: Zhang, Jun
Institution: Human BioMolecular Research Institute, San Diego, CA
Funding Agency: California Tobacco-Related Disease Research Program
Project ID: 9KT-0228
Project Funding Period: 1 July 2000 – 30 June 2003

Abstract: Smoking is a chronic condition that affects more than 46 million Americans. People who smoke are at risk of heart disease, cancer, and other tobacco-related illnesses that cost more than $50 billion annually to treat, and an additional $47 billion in indirect costs from lost time at work and disability. Cigarette smoking during pregnancy not only damages the health of the smoker, increases the incidence of spontaneous abortion and low birth weight, but also causes long lasting effect such as learning disabilities and hyperactivity in the offspring. Even though the rate of smoking in pregnant women is much lower than the general population in the United States, there were still over 400,000 women who smoked during their pregnancy in 1996, endangering their own health and that of their children. This number will possibly increase in the years to come due to the increasing smoking rate among teenage girls and young women. This is also an important problem in the rapidly growing state of California.

How nicotine affects the health of the fetus is a question that remains to be answered. We hypothesize that nicotine exposure during pregnancy can enhance a cell suicide program, called apoptosis, in developing neuronal cells of the fetus. The purpose of this proposal is to understand whether immature neurons in fetus are more sensitive to nicotine treatment as compare to mature neurons in adults; how the neurons commit to apoptosis after nicotine exposure; and whether we can use apoptosis inhibitors to stop this process. We will also study whether nicotine itself is the most potent reagent causing the neuron damage, or if nicotine

metabolites, the products generated in human body from nicotine after its consumption, can also harm neurons. Finding from this study will provide information for the future scientific research on the long-term effect of maternal smoking on the offspring. The results can be also applied to educational efforts for the general public and pregnant women to stop smoking.

Title: Cigarette Smoking or PCBs Effects on Estradiol
Principal Investigator: Zhu, Bao T.
Institution: University of South Carolina at Columbia, Columbia, SC
Funding Agency: National Cancer Institute
Project ID: CA074787
Project Funding Period: 1 May 1997 – 30 April 2001

Abstract: Earlier studies indicated that cigarette smokers have a decreased risk of uterine endometrial cancer and an increased risk of osteoporosis which are thought to be caused by an inhibitory effect of cigarette smoking on estrogen action. Some but not all studies have indicated that cigarette smokers have lower plasma levels of estradiol and enhanced systemic 2-hydroxylation of this estrogen. A major focus of this proposal is to characterize the effects of cigarette smoking on NADPH-dependent metabolism of estradiol to multiple hydroxylated and keto metabolites by human liver and, in particular, by extrahepatic estrogen target organs such as uterine endometrium and placenta. In addition, we will characterize the profile of multiple estradiol metabolites formed by placentas from women exposed accidentally to polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans which are potent inducers of microsomal monooxygenases. Our proposed studies should not only enhance our understanding of the antiestrogenic effect of cigarette smoking in women, but they should also provide us with useful information on the inducibility of cytochrome P450 enzymes that catalyzed the formation of various estradiol metabolites (including hormonally- active and/or genotoxic metabolites) in human liver as well as in human target organs for estrogen action. We plan to: (l).Determine the profile of NADPH-dependent estradiol metabolites formed by uterine endometrial microsomes from cigarette smokers and matched nonsmokers. (2).Determine the profile of NADPH-dependent estradiol metabolites formed by placental microsomes from cigarette smokers and matched nonsmokers. (3).Determine the profile of NADPH-dependent estradiol metabolites formed by liver microsomes from cigarette smokers and matched nonsmokers. (4).Determine the profile of NADPH-dependent estradiol metabolites formed by placental microsomes from women exposed accidentally to polychlorinated biphenyls and polychlorinated dibenzofurans. (5).Determine the effects of selective inhibitory antibodies to various cytochrome P450 isoforms on the pathways of estradiol metabolism observed in the studies described above. (6).Determine the effects of cigarette smoking on the concentration of unmetabolized estradiol in human uterine endometrium.