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AIM 2000

THE SHERATON
San Diego Marina
1380 Harbor Island Drive
San Diego, CA 92101
(619) 291-2900

Tobacco-Related Disease Research Program
5th Annual Investigator Meeting

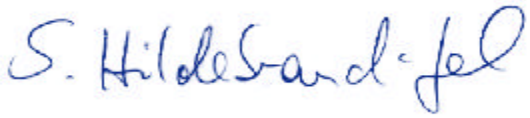
AIM 2000
ENVIRONMENTAL TOBACCO SMOKE: DYING WITHOUT TRYING?

Welcome From the Director

On behalf of the Tobacco-Related Disease Research Program staff and the University of California, I would like to extend a warm welcome to you to TRDRP's 5th Annual Investigator Meeting. Our theme for the plenary session is *ENVIRONMENTAL TOBACCO SMOKE: Dying Without Trying?* In this session, Drs. Slotkin, Reynolds, and Connolly will examine the biological and epidemiological evidence for the harmful effects of ETS and the policy implications of these findings.

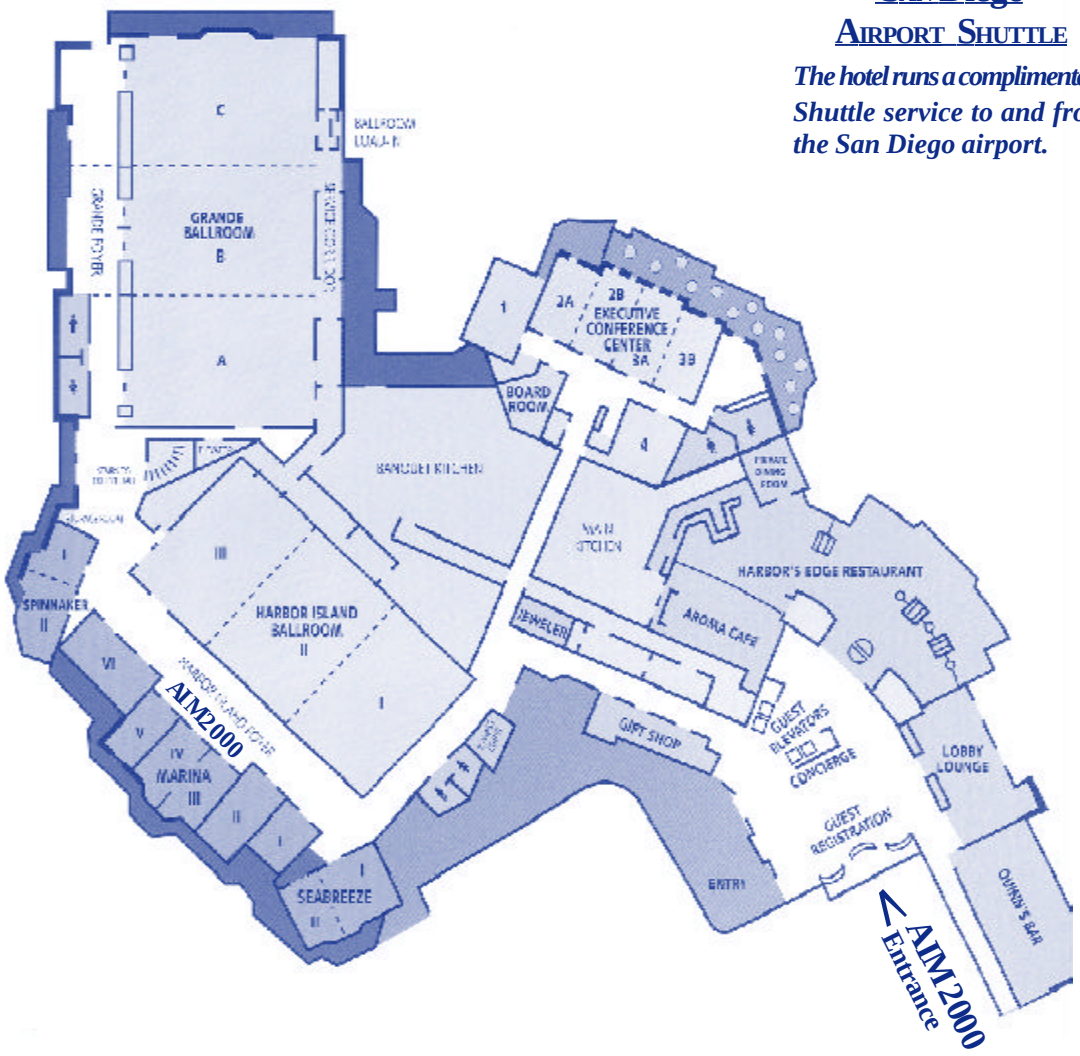
It has been a very productive year for TRDRP-funded research and a record 105 posters will be presented on Friday afternoon. I would like to encourage you to take a look at posters in areas outside of your own expertise to see what advances are being made in other areas to combat the detrimental effects of tobacco use.

I hope that this meeting will highlight the advances made in tobacco research, that it will provide ample opportunity for investigators to talk to each other, and that it will stimulate discussions about the pressing research questions remaining and how we might collectively tackle them.



Susanne Hildebrand-Zanki, Ph.D.

THE SHERATON SAN DIEGO Marina HOTEL



SAN Diego AIRPORT SHUTTLE

The hotel runs a complimentary Shuttle service to and from the San Diego airport.

ONSITE PARKING

NIGHT = $12.00

VALET PARKING

NIGHT = $18.00

AIM 2000 SCHEDULE OF EVENTS

November 30th, THURSDAY

8:00 am - 9:00 pm
REGISTRATION
(*Posters can be set up after 6:30 pm)*

CONCURRENT WORKSHOP SESSIONS
8:30 am - 5:00 pm
MARINA VI
Neuronal Nicotinic Receptors: Basic Mechanisms to Function
Maryka Quik and Darwin Berg

2:00 pm - 4:00 pm
MARINA V
Advances in Atherosclerosis Research
American Heart Association - Western States Affiliates

2:00 pm - 5:00 pm
MARINA IV
Youth and Young Adult Tobacco Cessation…Innovating for the Future
American Cancer Society - California Division, Inc.

MARINA II
Evaluating Progress in Reducing Exposure
to Secondhand Smoke in California
Department of Health Services - Tobacco Control Section

6:00 pm - 8:00 pm
LANAI GRASS
RECEPTION

AIM 2000 SCHEDULE OF EVENTS

DECEMBER 1st, FRIDAY

7:00 am -

REGISTRATION

7:00 am - 9:00 am

CONTINENTAL BREAKFAST & POSTER SET UP

9:00 am - 12 noon

HARBOR ISLAND I

PLENARY SESSION

Theodore Slotkin, Ph.D. - Duke University

Tobacco, Nicotine and Fetal Brain Damage: The Smoking Gun in ADHD and SIDS

Peggy Reynolds, Ph.D. - California DHS

ETS and Lung Cancer: The Epidemiologic Evidence

Gregory Connolly, D.M.D., M.P.H. - Mass. DPH

ETS Health Risks - Policy Implications

12 noon - 1:00 pm

HARBOR ISLAND II

LUNCHEON

POSTER SESSIONS

1:30 to 2:30 - Poster Sessions A-C

A) Policy - *Seabreeze II*
B) Pulmonary Diseases - *Marina VI*
C) California Multicultural Communities - *Marina I*

2:15 to 3:15 - Poster Sessions D-F

D) Epidemiology - *Seabreeze I*
E) Cancer: Mechanisms, Diagnosis and Treatment - *Marina III*
F) Community and Clinical Interventions - *Marina V*

3:00 to 4:00 - Poster Sessions G-I

G) Environmental Tobacco Smoke - *Marina VI*
H) Cardiovascular/Reproductive Health Effects - *Marina IV*
I) Nicotine Dependence - *Marina II*

AIM 2000 WORKSHOPS

Neuronal Nicotinic Receptors: Basic Mechanisms to Function

Organizers

Maryka Quik and Darwin Berg

1. **Nicotinic receptor synaptic and signaling mechanisms –** *Chair, Ken Kellar*

8:30 - 8:40 ***Introductory remarks***
Ken Kellar, Georgetown University

8:40 – 9:05 ***Calcium transients and gene regulation driven by nicotinic receptors on somatic spines***
Darwin Berg, University of California San Diego

9:05 – 9:30 ***Role of pre- and postsynaptic nicotinic receptors in ganglionic synaptic transmission***
Peter Sargent, University of California San Francisco

9:30 – 9:55 ***The myth of long lasting a4b2 nicotinic receptor inactivation***
Bruce Cohen, University of California Riverside

9:55 – 10:25 ***Coffee Break***

2. **Genetic manipulation to identify nicotinic receptor function –** *Chair, Jon Lindstrom*

10:25 - 10:35 ***Introductory remarks***
Jon Lindstrom, University of Pennsylvania

10:35 – 11:00 ***Genetic dissection of nicotine addiction***
Steve Heinemann, Salk Institute, San Diego

11:00 – 11:25 ***a7 Receptor mutants in oocytes***
Ricardo Miledi, University of California Irvine

11:25 – 11:50 ***Dopaminergic pathology and increased anxiety in mice with hypersensitive a4 receptors***
Henry Lester, California Institute of Technology

12:00 – 1:30 ***Lunch***

schedule continued on page 8

Neuronal Nicotinic Receptors: Basic Mechanisms to Function -continued

3. Nicotinic receptor mediated effects on development and degeneration – Chair, Ron Lukas

1:30 – 1:40 ***Introductory remarks***
Ron Lukas, Barrows Neurological Institute

1:40 – 2:05 ***Nicotinic receptors and nigrostriatal degeneration***
Maryka Quik, The Parkinson's Institute

2:05 – 2:30 ***Highly selective neural degeneration in brain induced by nicotine***
Gaylord Ellison, University of California Los Angeles

2:30 – 2:55 ***Nicotinic receptors regulate glutamate synapse development in auditory neocortex***
Raju Metherate, University of California Irvine

2:55 – 3:30 ***Coffee Break***

4. Mechanisms of nicotine addiction – Chair, Allan Collins

3:30 – 3:40 ***Introductory remarks***
Allan Collins, University of Colorado, Boulder

3:40 – 4:05 ***The development of nicotine reinforcement***
Francis Leslie, University of California Irvine

4:05 – 4:30 ***Neurobiological substrates of nicotine addiction***
Athina Markou, Scripps Research Institute

4:30 – 4:55 ***Advances in neuronal nicotine receptor research: implications for understanding and treating tobacco addiction***
Jack Henningfield, Pinney Associates, Inc., Bethesda

AIM 2000 WORKSHOPS

AMERICAN CANCER SOCIETY – CALIFORNIA DIVISION, INC.

Youth and Young Adult Tobacco Cessation…Innovating for the Future

Moderator
John Simmons, M.D.
Past President, ACS-California Division;
Senior Physician & Medical Oncologist, Kaiser Medical Ctr., Walnut Creek, CA

2:00 – 2:10	***Introductory Remarks***
2:10 – 2:55	***Youth Cessation – What do the Data Show?*** Art Farkas, Ph.D. Assistant Adjunct Professor University of California, San Diego
2:55 – 3:05	***Questions and Answers***
3:05 –3:50	***What Motivates Teens to Quit?*** Elizabeth Reibling Doctoral Student University of California, Irvine
3:50 – 4:00	***Questions and Answers***
4:00 – 4:45	***Youth Cessation Treatment*** Jack Hollis, Ph.D. Senior Investigator Center for Health Research, Kaiser Permanente
4:45 – 4:55	***Questions and Answers***
4:55 – 5:00	***Closing Remarks***

AMERICAN HEART ASSOCIATION – WESTERN STATES AFFILIATE

Advances in Atherosclerosis Research

Moderator

Fredric B. Kraemer, M.D.
President-elect, American Heart Association, Western States Affiliate
Member, TRDRP Scientific Advisory Committee
Associate Professor, Stanford University School of Medicine

2:00 – 2:05	***Welcome*** ***Fredric B. Kraemer, M.D.***
2:05 – 2:30	***Putting the risk in cardiovascular risk-benefit assessment*** Beatrice A. Golomb, M.D., Ph.D. Assistant Professor University of California, San Diego
2:30 – 3:00	***Apolipoprotein E in Atherosclerosis*** Linda K. Curtiss, Ph.D. Professor The Scripps Research Institute
3:00 – 3:30	***The Role of the Monocyte/Macrophage in Atherogenesis*** Daniel Steinberg, M.D., Ph.D. Professor of Medicine University of California, San Diego
3:30 – 4:00	***Tissue Factor and Cardiovascular Disease*** Nigel Mackman, Ph.D. Associate Professor The Scripps Research Institute

AIM 2000 WORKSHOPS

CALIFORNIA DEPARTMENT OF HEALTH SERVICES
Tobacco Control Section

Evaluating Progress in Reducing Exposure to Secondhand Smoke in California

Moderator
Jon Lloyd, M.A.
Chief of Data Analysis and Evaluation, Tobacco Control Section

2:00 – 2:10 ***Introductory Remarks***

2:10 – 2:55 ***Breathing Easier in California: Results from the California Tobacco Surveys***
Elizabeth A. Gilpin, M.S., Clinical Professor of Biostatistics
Cancer Prevention and Control Program, UCSD Cancer Center

2:55 – 3:05 ***Questions and Answers***

3:05 – 3:50 ***Why Are Californians Breathing Easier? Community-Level Results from the Independent Evaluation of the California Tobacco Control Program***
Beth Howard Pitney, Ph.D., Senior Research Scientist
Stanford University

3:50 – 4:00 ***Questions and Answers***

4:00 – 4:25 ***Findings from the California Bar-Patron Survey Data***
Hao Tang, M.D., Research Scientist
Tobacco Control Section

4:25 – 4:50 ***The Economic Impact of the Smokefree Bar Law in California***
David W. Cowling, Ph.D., Research Scientist
Tobacco Control Section

4:50 – 5:00 ***Closing Comments***
Jon Lloyd

Plenary Session Speaker Biographies

Gregory Connolly, D.M.D., M.P.H.

Director of the Massachusetts Tobacco Control Program

Gregory Connolly is the Director of the Massachusetts Tobacco Control Program. He is a graduate of Holy Cross College, Tufts Dental School, and Harvard School of Public Health. He is recognized as a national expert on smoking and is credited with passage of the 1986 federal law on smokeless tobacco. He has testified before committees of the U.S. House and Senate on numerous occasions. He has published over fifty scientific articles on this topic and was awarded the Surgeon General's Medallion for National Leadership by Dr. C. Everett Koop in 1987. He is a U.S. appointee to the World Health Organization's expert panel on smoking and health and has helped train public health officials in Asia and Eastern Europe to control tobacco use.

The Massachusetts Tobacco Control Program is a $48 million dollar state agency started in 1993. Since its inception, overall cigarette consumption has fallen 35%, almost four times the nation average. Adult smoking rates have fallen 22% and the adolescent smoking 25%. The results of the Massachusetts Tobacco Control Program indicate large-scale campaigns that combine media, price increases, and promotion of clean indoor air can greatly reduce cigarette consumption.

Peggy Reynolds, Ph.D.

Cancer Epidemiologist, Environmental Health Investigations Branch, California Department of Health Services

Peggy Reynolds, Ph.D. is a cancer epidemiologist in the California Department of Health Services' Environmental Health Investigations Branch, and currently serves as the chief of the Environmental Epidemiology and Geographic Information Section. She received her Ph.D. in Epidemiology from the University of California at Berkeley and spent several years as an epidemiologist for the California Tumor Registry and San Francisco Bay Area SEER (Surveillance, Epidemiology and End Results) program. She has conducted a number of cancer epidemiology studies, with a particular focus on environmental risk factors. Dr. Reynolds was a co-investigator for the U.S. Multicenter Study of Environmental Tobacco Smoke and Lung Cancer in Non-Smoking Women, which is the largest such study of non smoking women published to date and one of the landmark studies in this area. She has published a number of papers on ETS and lung cancer and served on the 1998 U.S. OSHA-sponsored ETS Risk Assessment Workshop.

Plenary Session Speaker Biographies

THEODORE SLOTKIN, PH.D.

Professor, Department of Pharmacology & Cancer Biology, Department of Psychiatry & Behavioral Sciences, Department of Neurobiology, Duke University Medical Center

Dr. Slotkin received his Ph.D. in Pharmacology & Toxicology from the University of Rochester in 1970. He has done extensive research in the areas of developmental pharmacology and toxicology, neuropharmacology and neurochemistry, and cell differentiation and growth regulation. His research is aimed toward understanding the interaction of drugs, hormones and environmental factors with the developing organism, with particular emphasis on the fetal and neonatal nervous system. His most notable achievements center around the effects of fetal exposure to drugs of abuse, especially tobacco and nicotine. He has received numerous honors and awards for his research work, notably the Alton Ochsner Award Relating Smoking and Health, and the Otto Krayer Award in Pharmacology, and has published over 360 peer-reviewed articles. He has served on NIH Consensus Panels on Pharmacotherapies for Smoking Cessation During Pregnancy and on the Use of Antenatal Steroids, has chaired reviews for the California Tobacco-Related Diseases Research Program, is currently appointed to the National Board of Medical Examiners, and serves on the editorial boards of numerous scholarly journals.

A1

Reducing provision of tobacco to minors from social sources

Fortmann, Stephen P.
Stanford Center for Research in Disease Prevention

California's teen smoking rate remains high even though the rate of illegal tobacco sales to minors declined significantly over the last three years. Some researchers have suggested that smoking prevention efforts may benefit from targeting nonretail sources of tobacco, such as friends, family, or strangers. This field demonstration study evaluated a school-based intervention designed to reduce the availability of tobacco from social sources by changing personal and social norms about giving or buying cigarettes for youth. A concurrent merchant education campaign was conducted to complement these efforts to reduce youth access.

Two rural communities in northern California, matched on population size, ethnic representation, and number of tobacco outlets, were assigned to treatment or control. In the treatment community, 8th graders were exposed to a two-hour curriculum and a communication campaign comprised of letters mailed to students and their parents, posters at school, and t-shirts distributed at a school event with California's Team O2 Van. In addition, study staff visited all tobacco retailers twice to provide employee training materials with corrective feedback from undercover purchase attempts.

Evaluation data for the school-based intervention were derived from anonymous, in-class surveys conducted in May 1999, nine months before the intervention, and June 2000, one month after. Thus, the study sample was comprised of two independent cross-sections of 8th graders in the treatment community (pre-test n=270, post-test n =275) and control community (pre-test n=211, post-test n=190). The sample was 57% white, 22% Latino, 21% other or multiple ethnicities, and included slightly more girls (51%) than boys. Multiple linear and logistic regression analyses were used to test for intervention effects on perceived access to tobacco from other teens, seeing social source exchanges in the past 30 days, and intentions to give or buy cigarettes for others. For outcomes measured before and after the intervention, a significant interaction between time and community indicated an intervention effect. For outcomes measured only on the post-test, intervention effects were inferred from significant differences between the two communities.

Approximately two-thirds of 8th graders in the treatment community remembered receiving the letter, recalled seeing the posters, and wore the t-shirt that communicated intervention messages. Students were significantly more likely to discuss and disapprove of social sources in the treatment than the control community. However, self-reported exposure to social source exchanges decreased similarly in both communities. In addition, the intervention did not change students' perceptions about access to tobacco from social sources nor intentions to give or buy cigarettes for others.

Evaluation data for the merchant intervention were derived from undercover buys conducted before and after the merchant education campaign. Female confederates (ages 16-17) attempted to purchase cigarettes from all tobacco retailers in the treatment (n=20) and control community (n=16). Although the sales rates decreased in both locations, a larger decrease was observed in the treatment (30.2% vs. 16.9%) than control community (20.4% vs. 14.6%).

A2

Promoting compliance with smokefree schools: changing social norms

Emery, Sherry / Spitchley, Trisha
University of California, San Diego
Department of Family and Preventive Medicine

Background: School smoking policies can discourage adolescent smoking by making smoking inconvenient and officially recognized as an undesirable activity. In 1998, the San Diego City School District (SDCS) implemented one of the most aggressive approaches in California and the US for achieving a smoke-free learning environment. Students face disciplinary action—including possible suspension—for violating the policy. At the discretion of the principal, suspensions may be waived if students sign a contract and attend a smoking cessation program. Therefore, effectively, most students who violate the policy must attend these cessation classes. However, enforcement of the policy across schools and among individual students has been inconsistent and created unforeseen consequences.

Methods: Structured interviews with school administrators, teachers, and school police, as well as focus groups with students in 4 of the district's 12 traditional high schools, plus 2 alternative high schools were used to identify a) barriers to enforcement of and compliance with the SDCS smokefree schools policy, and b) opportunities and strategies to overcome these barriers, and c) related issues.

Results: High school principals received little to no instruction about the goals of the policy and implications of its consistent enforcement. Many use their discretion in enforcing the policy to discipline troubled adolescents, but place a low priority on enforcing the policy among high achieving students and athletes. Few administrators involve parents in the enforcement process.

Conclusions: An administrator in-service or education component might increase the level of priority principals place on consistently enforcing the policy. Contacting parents with information about ways they might help reduce the chances of their teen becoming a lifelong smoker, such as implementing home bans and monitoring teens' smoking and risky behaviors, may also contribute to policy compliance and reduce adolescent smoking.

POSTER SESSIONS
Session A: Policy

A3
Youth-driven tobacco policy change: a cross-site analysis

Constantine, Norm / Dennis, Denice A.
WestEd, School and Community Health Research Group
Contra Costa Health Services

With funding from the California Department of Health Services Tobacco Control Section, TIGHT was created in 1997 through Contra Costa County Health Services. TIGHT is a youth-driven project to: (1) develop teams of youth with the capacity to recognize and counter tobacco industry targeting in their communities; (2) work with policy and decision makers to develop and implement effective ordinances and policies to reduce youth access to tobacco; and (3) develop youth leadership skills (e.g., public speaking, problem solving, action planning) and enhance resilience. TIGHT's organizational structure is designed to maximize youth development. To date, TIGHT has been successful in passing county-wide and 13 individual city tobacco-free youth ordinances. Evaluation data suggest that the program has been successful in supporting the positive development of participating youth.

This first-year pilot study was originally conceptualized as preparatory to our submission of a three-year community-academic research award (CARA) application. The ultimate goals of the three-year study were to: (1) document and better understand the dynamics and results of youth activism in changing local tobacco policies; (2) identify positive effects on the youth activists; and (3) analyze the experience of implementing TIGHT across five selected Contra Costa County communities.

Focusing on one selected city within Contra Costa County, during this pilot study we developed and tested data systems strategies to track and analyze (1) tobacco related community actions and changes; (2) media coverage; and (3) developmental supports (*caring relationships, high expectations, and opportunities for meaningful participation*) provided to youth participants. We also conducted pilot interviews with policy makers, other local community members, and TIGHT youth participants on the perceived benefits and challenges of TIGHT as a youth advocacy initiative; and consultant-facilitated focus groups with research staff and with program staff to explore collaboration issues, challenges, and successes.

The results of this study will inform efforts that foster youth leadership and policy change to prevent and reduce tobacco use among youth. These results will also be used to produce support materials to assist other communities in locally replicating this program.

A4
Differential effects of a tobacco tax increase on latino vs. general population health in California

Kaplan, Robert M.
University of California, San Diego

Background: In January of 1999 California enacted Proposition 10, an initiative to raise the state excise tax on cigarettes by $0.50/pack. We compared the expected impact of this tax increase on the health of the Latino population in California with its effect on the health of the overall state population.

Method: We identified a range of estimates of the price elasticity of demand for cigarettes in the general US population and specifically among Latinos. We used these elasticity estimates to calculate changes in overall and in Latino smoking prevalence in California that would likely result from a range of actual and proposed cigarette price increases. Using Latino-specific smoking prevalence and mortality data for California, we then associated these projected changes in smoking with changes in health status, using a method that combines morbidity and mortality into a common index of health status: Quality Adjusted Life Years. To estimate the morbidity consequences of smoking, we used the Health and Limitations Index (HALex) measure of years of healthy life from the 1994 National Health Interview Survey. Since small numbers of NHIS Latino respondents in several age groups made Latino HALex scores relatively unstable, we applied general population HALex scores to both the Latino and overall groups for our final estimates. We are continuing to investigate the feasibility of using Latino-specific QALY measures such as an aggregate measure derived from a multiple year NHIS data set.

Results: For our base case of an additional $0.50/pack tax and a Latino price elasticity of demand of -1.0, Latino QALYs saved per smoker would be more than double QALYs saved per smoker for the entire state population in the first year after the tax increase and proportionally even higher 75 years later. QALYs include two components: life expectancy and quality of life. For the state population as a whole about two thirds of the increase in QALYs was attributable to quality of life. For the Latino population about 80% of the increase was attributable to the quality of life component.

Conclusions: The California Latino population may receive a proportionally larger share of the health benefit resulting from a tobacco tax increase then the state population as a whole. This raises a challenge to the usual argument that tobacco excise taxes, or tax increases, are necessarily regressive, since lower-income and minority populations are likely to experience a relatively greater health benefit. Such results may inform current World Health Organization and the World Bank discussions recommending cigarette tax increases as a central instrument of tobacco control. Our finding is conditional, however, on assumptions of a higher Latino demand elasticity, as well as on the quality-of-life measurement methodology used.

A5

The Role of changes in cigarette prices in smoking uptake over time

Emery, Sherry / White, Martha
University of California, San Diego / UCSD Cancer Center

Background: It has been stated that raising cigarette excise taxes may be the single most important tool for deterring adolescent smoking. However, previous research suggested that experimenters might not be sensitive to cigarette prices. It is important to understand how and whether experimenters respond to higher cigarette prices because many will go on to become regular smokers and for some, the first few cigarettes are enough to trigger a vulnerability to nicotine dependence. If cigarette taxes do not influence this group, it is important to reach them with other tobacco control policy tools. Longitudinal data can overcome some of the limitations in the cross-sectional studies to provide an improved understanding of the relationship between price and experimentation.

Methods: Using data from the longitudinal 1989-1993 Teen Attitudes and Practices Surveys (TAPS), we analyzed the probability of making transitions in smoking uptake as a function of change in cigarette prices over time and relevant baseline demographic and psycho-social variables.

Results: Neither baseline prices nor changes in cigarette prices over the 4-year period affected transitions from never smoker status to experimenter. Baseline prices were not associated with transitions from never smoker to current or established smoker, but changes in cigarette prices were negatively associated with the transition from never smoker to current smoker (elasticity of price change = -0.58; p=0.05), and from never smoker to established smoker (elasticity of price change = -1.46; p=0.01). The effect of changes in price on transitions from experimenter to established smoker were modest (elasticity of price change =-0.21; p=0.05); this transition was not influenced by baseline prices.

Conclusions: Higher excise taxes may not deter experimentation, but may deter progression to established smoking. The absolute level of cigarette prices may be less important in adolescents' transitions in smoking uptake than is the size of the increase of cigarette prices during adolescence.

A6

Cost of smoking in an adult woman of reproductive age

Lightwood, James M.
University of California, San Francisco

This project concerns the economics of cigarette smoking cessation in the general population. This abstract reports results for smoking related reproductive costs in adult women.

Maternal smoking during pregnancy causes low birth weight and maternal complications of pregnancy. Environmental tobacco smoke from maternal smoking after birth increases the occurrence of childhood respiratory and middle ear disease. Estimates of the resulting total excess direct medical costs (interpreted as expenditures received for treatment) are large: about $1.2 billion annually. However, existing cost estimates provide little guidance in determining how much should be allocated towards smoking prevention and cessation in a young adult woman. Existing estimates are inadequate because they concern the tobacco related costs for a particular condition only, while the unit of treatment for smoking prevention is the individual patient, and the relevant costs are all smoking related costs occurring in that individual over a given period of time.

This poster presents the first estimates of the total reproductive costs of smoking per individual woman smoker. Estimates were calculated for White and Black women for the costs of smoking related low birth weight, spontaneous abortion, ectopic pregnancy, maternal complications of delivery, childhood respiratory illness, and otitis media. The excess risks of disease and costs due to smoking were estimated using published data. Lifetime reproductive costs per smoker were calculated using data on the expected number of pregnancies and live births for a typical U.S. woman between her 18th and 44th birthday.

The total undiscounted costs for a woman who smokes continuously from age 18 to 44 are $2,507 and $4,475 for a White and Black woman, respectively. Approximately half of the costs are incurred in the first ten years (ages 18 to 27): $1,750 and $2,368, respectively. When expected smoking cessation is included, total costs are lower because a smoker may quit before her 44th birthday. Including expected smoking cessation, the total undiscounted costs to age 44 are $1,427 and $2,249 for a White and Black woman respectively.

Using a 2.5% discount rate, the total present value discounted costs for a continuous smoker to age 44 are $1,919 and $3,545 for Whites and Blacks, respectively. The cumulative cost up to age 27 is $923 and $2,127, respectively. When expected future smoking cessation is included in the analysis, the discounted costs to age 44 are $1,123 and $1,844, respectively.

These estimates imply that society could spend significant resources in order to prevent a typical 18 year old woman from smoking, or to quit before establishing a significant smoking history, and still save money: up to $1,123 for White and $1,844 for a Black woman, respectively. For women who are expected to smoke continuously until the age of 44, the amounts are $1,919 and $3,545, respectively. Future research will concern other aspects of smoking cessation: savings from cancers and other respiratory diseases that are prevented following cessation, and the net savings from all major smoking related diseases after the cost of smoking cessation is taken into account.

POSTER SESSIONS
Session A: Policy

A7
The public health impact of changes in smoking behavior: results from the tobacco policy model

Tengs, Tammy O.
University of California, Irvine

Interventions and policies designed to reduce tobacco use differ according to the population they are intended to reach and the nature of the behavior they are intended to alter. Thus, their ultimate impact on the nation's health will vary. The objective of this study is to compare the public health gains from preventing initiation, encouraging cessation, or avoiding relapse in different genders and at different ages.

To estimate public health gains, we developed a computer simulation model called the Tobacco Policy Model. The Model is designed to calculate gains in quality-adjusted life-years (QALYs) to the U.S. population given any change in tobacco use. The population is divided into cohorts according to age, gender and smoking status. Transitions such as aging, birth, death, smoking initiation, cessation, and relapse are assumed to occur annually. Transition probabilities are based on estimates from publicly available databases and can vary by age, gender, smoking status, and year.

The Tobacco Policy Model includes several innovative features that improve on historical models: First, we model changes in smoking behavior rather than prevalence. Second, we capture not only gains in survival, but also improvements in health-related quality of life. Third, we simulate the effects of tobacco use reduction over multiple generations. Fourth, we build into the model the recognition that not all of the mortality and morbidity differential between smokers and non-smokers can be attributed to smoking.

To assess the health gains from different forms of smoking behavior change in different ages and genders, we simulated a 10% reduction in the annual probability of initiation vs. a 10% increase in cessation or a 10% reduction in relapse in males and females in six age groups: 10-19, 20-29, 30-39, 40-49, 50-59 and 60-69.

Results indicate that the relative value of preventing initiation, encouraging cessation and averting relapse differs by age and gender. Among youth and young adults, reducing initiation yields far more QALYs than encouraging cessation or averting relapse. In middle-aged adults, cessation yields the most QALYs, followed by averting relapse and reducing initiation. In the oldest age group, averting relapse yields the most QALYs followed by cessation and reducing initiation. Changing cessation and relapse is more beneficial in males than in females while reducing initiation is more beneficial in females. Comparing all scenarios, reducing initiation in youth is likely to offer the largest public health impact over the next century – yielding on the order of 6-7 times more QALYs than getting older smokers to quit.

A8
Tobacco industry documents and the need for standards

White, Celia
University of California, San Francisco

In 1998, the Master Settlement Agreement stipulated that each tobacco company create and maintain an online digital archive of documents produced in the litigation. These sites are slated to continue until 2008.

There are six industry document websites—Philip Morris, RJ Reynolds, Brown & Williamson. Lorillard, the Tobacco Institute and the Council for Tobacco Research. While each provides the same service—searching and retrieval of the records, and the viewing of digital images of each document—the interfaces, searching functionality, and image formats vary. Sometimes these variations are quite extreme, forcing the user to adjust to a completely different interface and to develop new search strategies and skills in order to retrieve consistent results. Other variations are so subtle that only a thorough studying and memorization of the help documentation will elucidate the accepted technique.

This poster illustrates some of the differences between the sites—search functionality, image formats, and variations in record fields. The present community of tobacco document researchers is outlined, and an assessment of their needs is given.

This needs assessment segues into a discussion of the obvious need or standards for the data structure and indexing of tobacco documents. UCSF's work in the application of accepted standards is described, as well as new work creating standards and defining best practices for working with tobacco industry documents. Such work includes the UCSF-TDO Tobacco Citation Field Definitions, the UCSF-ANRF Thesaurus, Best Practice for Indexing, application of metadata standards such as Dublin Core, Encoded Archival Description and XML, and the exploration of appropriate image formats for document provision in an online environment.

The application of existing standards will greatly enhance use of the documents, and such use will increase their value to the present community. The development and implementation of standards specific to this unique set of digital information will greatly improve upon present access mechanisms and will in turn increase the potential for document research and use beyond the present scope.

A9

Genetic research on smoking: foreseeable applications and policy issues.

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Goal of the project: Genetic research on smoking, in particular susceptibility to nicotine addiction, is currently underway with the hope that this will lead to innovative approaches to prevention and cessation. This emerging body of research will raise important ethical, social and policy issues. How will an understanding of smoking in genetic terms influence policies to reduce tobacco use, medical approaches to smoking cessation, and societal attitudes toward smoking and smokers?

Work performed to date: With this IDEA planning grant, investigators at the Stanford Center for Biomedical Ethics performed a multidisciplinary literature review, established collaborations with genetic researchers, and developed qualitative instruments to single out issues and refine research questions to be included in a more comprehensive research proposal. In phase one of this pilot research, we conducted interviews with 13 experts on smoking determinants/tobacco control/health policy to assess the current status of genetic research, its foreseeable applications and the potential impact of genetic explanations.

Results: Acknowledging the complex interplay between biological, behavioral and environmental determinants of tobacco use, experts' views about the genetic contribution to smoking converged toward the following scenario: It will take 10 years or more before genetic-based interventions are developed; many genes with weak effects will be identified instead of "a gene" with strong effects; the genes will most likely play a role in addiction and in the inability to quit, rather than in the initiation of smoking. A majority of experts believe that preventive genetic testing, i.e. testing an identifiable population such as adolescents, and then targeting preventive activities to those individuals most susceptible, was highly improbable. In contrast, tailoring cessation treatments using pharmacogenetics (i.e. drug development based on gene identification) was seen as the most likely and useful application.

Most experts recognize the power of genetic explanations to influence how government perceives its responsibilities in preventing smoking-related diseases. One positive impact is that genetic understanding might further "medicalize" smoking, leading to increased health insurance coverage for cessation treatments. The downside is that providing a biological explanation for why people smoke might be viewed as fatalism, making people more resistant to preventive messages. Some suggest that the eventual impact on public policy will depend on who controls the information and how it is presented to the public, raising concerns about the media and the tobacco industry. Almost unanimously, experts believe that the tobacco industry will try to use genetic explanations of smoking behavior as a way of shifting responsibility for nicotine addiction away from cigarettes and onto an individuals' genetic makeup.

Significance and future directions: Genetic studies of complex behavioral phenomena like smoking seek correlations between common biological differences and specific aspects of smoking behaviors. One immediate concern is that research findings could be used to single out and discriminate against socially or racially identified groups already labeled as more likely to smoke. It is thus imperative to pay close attention to the socio-cultural context which shapes research into the genetic epidemiology of smoking. Future ethical inquiry should also focus on the perspectives of pharmaceutical and health insurance companies, who will be key players in developing research applications.

B1
Quantification of lung doses from inhaled tobacco smoke

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The goal of this research is to examine the effects of the odd aerodynamic behavior of cigarette smoke on deposition in the human respiratory tract and on disappearance from the air of indoor environments. Fresh tobacco smoke clouds and wisps contain dense concentrations of microscopic particles - as many as several billion particles per cubic centimeter or air. Because of the high density of particles, this smoke can exhibit unusual behavior. Cigarette smoke clouds move with a collective or combined motion, rather than as distinct particles with independent motions

The research has three arms: smoke persistence in a room, deposition in airway models and mathematical modeling. Tobacco smoke has been studied in a room where stirring, temperature and humidity were controlled. An effective means of rapidly decreasing the airborne smoke was stirring the room air with a small (5 inch diameter) fan. Tobacco smoke concentration was also affected by changes in temperature or humidity with the fastest disappearance occurring at low temperature and low relative humidity. Introducing heated adult or child mannequins also decreased the persistence of the fresh tobacco smoke. This information is useful in designing strategies to eliminate smoke from environments where copious ventilation is impractical.

The unusual behavior of mainstream and sidestream tobacco smoke inhaled into humidified, warmed hollow models that are surrogates for human airways have been studied. Both mainstream and sidestream smoke exhibited unusually large deposition efficiencies in all these models compared to what is expected. The smoke particles behaved as if they are about ten times their actual size. These data were used to modify existing mathematical models of particle deposition in the human lung.

A modest difference in particle size of a carcinogenic component of sidestream tobacco smoke (benzo(a)pyrene) and whole sidestream smoke has been found. Based upon our mathematical models, this shift to smaller particle size leads to predicting a greater fraction of inhaled benzo(a)pyrene depositing than expected using sidestream tobacco smoke particle sizes. This theoretical prediction has been supported in replica hollow airway model deposition studies. These data can be used to refine the risk assessment from sidestream tobacco smoke.

A computer model of particle deposition in the human lung was modified for tobacco smoke by adding two new physical phenomena, cloud settling and wall effects. The resulting predictions for smoke deposition utilizing these phenomena were significantly different from predictions with the unmodified code. Although particle deposition predictions from the modified mathematical model were consistent with our hollow model results, it is clear that other phenomena will need to be modeled in order to obtain accurate predictions of cigarette smoke doses to the lung.

The data generated from this project are expected to help industrial hygienists evaluate and control tobacco smoke in indoor settings, and to aid risk-assessors in predicting the risks from inhaled environmental tobacco smoke for children and adults.

B2
Ceramide-induced apoptosis in lung epithelial cells is regulated by oxidative stress

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Cigarette smoke is known to contain abundant free radical species and reactive oxygen species (ROS), which affect lung epithelial cell function. Although a link between reactive oxidants and epithelial injury has been established, the cellular mechanisms involved are yet unknown. Our studies aim to determine the effects of exposure to reactive oxidants on lung epithelial cell growth and death processes. We proposed to investigate the cellular pathway of ceramide, a cell sphingolipid which regulates the process of programmed cell death (apoptosis) and to characterize the effects of reactive oxidants of tobacco smoke, such as H_2O_2, on ceramide signaling and the resulting apoptosis in the lung epithelium. We have hypothesized that ROS are mediators of lung injury and glutathione (GSH) is the major cell defense against oxidative stress.

We have recently shown that H_2O_2 induces ceramide-mediated apoptosis both in primate primary airway epithelial cells and in human A549 alveolar epithelial cells. H_2O_2 activates a neutral magnesium-dependent sphingomyelinase (nSMase) localized to the plasma membrane, suggesting that H_2O_2 induces hydrolysis of sphingomyelin to ceramide and generates the initial apoptotic signaling at the cell membrane. When the effects of H_2O_2 were measured directly on SMase activity, treatment of airway epithelial cells with 75 mM H_2O_2 induced a greater than 200 % activation of nSMase activity within 5-10 min, but not of acidic aSMase activity, suggesting that in airway epithelial cells only nSMase (membrane) and not aSMase (lysosomal) is activated by H_2O_2.

We have also demonstrated that exposure to GSH at physiological concentrations (1-10 mM) inhibits neutral sphingomyelinase (nSMase) but has no effect on acidic SMase (aSMase) activity from A549 human alveolar epithelial cells. In addition, treatment of A549 cells with GSH results in loss of their ability to produce ceramide, whereas depletion of intracellular GSH by DL-buthionine-[S,R]-sulfoximine (BSO) induces ceramide levels and apoptosis. Moreover, GSH blocks H_2O_2- and C6-ceramide-mediated induction of intracellular ceramide generation and apoptosis. These effects cannot be mimicked by oxidized GSH or other thiol antioxidants, such as dithiothreitol (DTT) and b-mercaptoethanol. Our results suggest that in lung epithelial cells, nSMase is inactive in the presence of physiological concentrations of GSH (1-10 mM) and thus, GSH depletion may be the link between oxidative stress and ceramide-mediated apoptosis.

We believe that further elucidation of the molecular mechanisms linking oxidative stress of tobacco smoke to apoptotic signaling pathways will improve pharmacological intervention in the signaling processes that regulate tobacco smoke oxidant-mediated diseases in the lung.

POSTER SESSIONS
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B3
Tobacco smoke-related acrolein modulates neutrophil properties.

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Cigarette smoking is known to exacerbate and contribute to various respiratory tract diseases characterized by chronic inflammatory-immune activation. Previous investigations have indicated that acrolein, a highly toxic unsaturated aldehyde in cigarette smoke, may be responsible for many of the cellular effects of cigarette smoke exposure. Indeed, cigarette smoke-derived acrolein was found to markedly inhibit the respiratory burst activation in neutrophils. In further studies, we observed that exposure of isolated human neutrophils to either gas-phase cigarette smoke or to acrolein (1-20 µM) resulted in dose dependent increases in overall tyrosine phosphorylation and in activation of mitogen-activated protein kinase pathways (ERK1/2, p38 MAPK), as determined by Western blotting with specific antibodies. Activation of these pathways is thought to control such processes as respiratory burst activation, granule secretion, generation of pro-inflammatory cytokines, and/or neutrophil survival or apoptosis. Indeed, acrolein exposure (10 µM) significantly increased the release of the chemoattractant interleukin (IL)-8 from neutrophils over 4-8 hrs, measured using an ELISA assay, and this was inhibited in the presence of SB203580, an inhibitor of p38 MAPK-dependent signaling pathways. Activation of p38 MAPK is often thought to be involved in promoting neutrophil apoptosis, but has also been suggested to inhibit spontaneous neutrophil apoptosis. Exposure of neutrophils to 1-10 µM acrolein was found to markedly reduce the rate of spontaneous apoptosis, measured by either annexin-V binding using flow cytometry or by a TUNEL assay. This inhibitory effect of acrolein was not significantly affected by inclusion of the p38 MAPK-inhibitor SB203580, suggesting that mechanisms other than p38 MAPK activation are involved in this response. The activation of caspases such as caspase-3 is commonly involved in the execution of apoptosis, and activation of caspase-3 in neutrophils was markedly inhibited by acrolein, presumably due to direct alkylation of its reactive cysteine residue. Indeed, the cellular effects of acrolein appeared to correlate with the extent of depletion of cellular GSH, which may be directly related to modifications of redox-sensitive protein cysteine residues. Collectively, our results suggest that cigarette smoke-related acrolein can markedly affect various neutrophil functions, which would contribute to prolonged and exacerbated inflammatory processes in the lung by increasing neutrophil recruitment and inhibiting neutrophil clearance by apoptosis. Indeed, chronic neutrophilia and delayed spontaneous neutrophil apoptosis appear to be a feature of chronic inflammatory lung diseases such as cystic fibrosis, and cigarette smoking or frequent exposure to environmental tobacco smoke could contribute to such alterations in phagocyte recruitment and regulation.

B4
Signaling mechanisms initiated by tobacco Smoke in the regulation of gene expression in human airway epithelium

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Our laboratory is examining the molecular events that occur after exposure of the airway epithelium to tobacco smoke. We are specifically interested in determining the signaling cascades that are responsible for inducing airway inflammation. We have previously demonstrated that tobacco smoke induces expression of the pro-inflammatory cytokine IL-8 and a squamous cell marker SPRR1 in human airway epithelial cultures. Because pro-inflammatory mediators and squamous cell metaplasia are postulated to contribute significantly to or be a marker of pulmonary diseases such as chronic bronchitis, emphysema, and lung cancer, we want to further examine the molecular mechanisms by which tobacco smoke increases IL-8 and SPRR1 production in human airway epithelial cells.

In our study, primary human tracheobronchial epithelial (TBE) cells were exposed to the gas phase of tobacco smoke for three minutes immediately followed by exposure to the soluble components of cigarette smoke for one and one half hours. Nuclear extracts were prepared and electrophoretic mobility shift assays (EMSA) were performed using ^{32}P-g-ATP end-labeled NF-kB or AP-1 consensus sequences. We observed that tobacco smoke induced a dose-dependent increase in AP-1 DNA-protein complex formation. There was no observable increase, however, in NF-kB activity, as measured by EMSA. To further explore this finding, we examined potential upstream events leading to AP-1 activation. Using a similar smoke-exposure strategy, whole cell extracts were collected for SDS-PAGE analysis to examine the presence or absence of phosphoylated mitogen-activated protein kinases (MAPK). MAPK pathways are known activators of several commonly known transcription factors, such as AP-1. Also, TBE cells were co-transfected with an AP-1 luciferase reporter gene and various MAPK inhibitors and exposed to cigarette smoke as above. In these experiments, we found that two MAPK, JNK-1 and ERK-2, appear to play a role in activating the transcription factor AP-1 in airway epithelial cells.

In the future, we plan to further elucidate the signaling pathways that are involved in airway epithelial cells after exposure to tobacco smoke. For example, we will examine the upstream events that lead to activation of MAPK specifically. Also, we plan to examine the changes in cigarette smoke-induced IL-8 and SPRR1gene expression when AP-1 activation or AP-1-DNA binding is blocked. By further understanding the molecular mechanisms of tobacco smoke-induced gene expression, we hope to gain insights into how smoking-related lung diseases are initiated and maintained. We may also be able to recognize early markers of tobacco-induced lung disease that allow the clinician to intervene before significant damage has occurred.

B5

Regulation of lung inflammation by the LTC_4 synthase pathway.

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Tobacco use has been demonstrated to increase the risk of bacterial lung infection. The current research has been performed to examine the effect(s) of the bacterial component, lipopolysaccharide (LPS), on leukotriene C_4 (LTC_4) synthase gene expression in mononuclear phagocytes. The formation of LTC_4 is the first committed step in the synthesis of the cysteinyl leukotrienes, LTC_4, LTD_4, and LTE_4. The cysteinyl leukotrienes are known to mediate a wide range of inflammatory responses. LPS has been demonstrated to elicit a localized proinflammatory response involving monocytes/macrophages that may promote host defense against bacterial infection. LPS exerts potent effects on monocytes/macrophages through the activation of the Toll-like receptors (TLR) and CD-14.

The monocyte-like cell line, THP-1, was screened for the presence of TLRs by RT-PCR and was found to express both TLR-2 and TLR-4. Conditioning of THP-1 cells with 1 µg/ml of E. coli 0111:B4 smooth LPS for 24 hrs resulted in a significant decrease in LTC_4 synthase mRNA, as detected by Northern blot analysis. Conditioning of cells with 0111:B4 smooth LPS and Salmonella minnesota Re 595 LPS at 10 ng/ml resulted in a lesser decrease in LTC_4 synthase mRNA, suggesting that the effect of LPS is dose-dependent. Time course Northern blot analyses demonstrate that the LPS-induced decrease in mRNA was time-dependent, resulting in suppression of mRNA as early as 16 hrs. Cycloheximide conditioning of LPS-treated THP-1 cells for 24 hrs did not inhibit the LPS effect on LTC_4 synthase mRNA, suggesting that synthesis of a new protein transcription factor was not required. Further work will attempt to address the possible mechanism(s) of action of LPS on the expression of the LTC_4 synthase gene and its products.

We conclude that LPS downregulates LTC_4 synthase gene expression in a dose and time-dependent manner in the monocyte-like cell line, THP-1. We suggest that the effect of LPS on LTC_4 synthase gene expression may have implications for monocyte/macrophage-mediated host defense against bacterial infection. In addition, our data may have important implications for the understanding of the effects of bacterial exposure in the pathogenesis of tobacco-related inflammatory lung diseases such as asthma and COPD.

B6

Effect of tobacco smoke on leukocyte adhesion in vivo

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The purpose of this investigation is to examine the effect of nicotine, a constituent of tobacco smoke on leukocyte trafficking in blood circulation, especially in the lungs. We utilized a dorsal skinfold chamber model of lung transplantation and examined the effect of nicotine on the dynamics of leukocyte (neutrophil or eosinophil)-endothelial interactions in lung microvessels by intravital microscopy. These results suggest that nicotine can function as a proinflammatory agent to induce vascular E-selectin and P-selectin expression in lung microvessels. Pre-treatment of animals with anti-P-selectin + anti-E-selectin antibodies resulted in the blockade of the ability of nicotine to induce leukocyte rolling and adhesion in lung microvessels. More importantly, treatment with anti-L-selectin + anti-E-selectin + anti-P-selectin antibodies resulted in complete inhibition of nicotine induced inflammation in pulmonary microvessels. In addition to the proposed aims, we have examined if nicotine inhibits hematopoieis in long term bone marrow cultures and trafficking of hematopoietic stem/progenitor cells (HSPC) under conditions of flow. We have embarked on these experiments because both bone marrow derived neutrophils and eosinophils are derived from HSPC and constituents of cigarette smoke including nicotine are more than likely to effect function and recruitment of these precursor cells. We therefore examined the effect of nicotine on bone marrow stromal cells as these cells are integral component of the hematopoietic environment and act as positive or negative modulators of HSPC. Our studies demonstrate that treatment of long term bone marrow cultures (LTBMC) with nicotine results in a dose dependent inhibition of stromal layer formation. Nicotine also delays the onset of hematopoietic foci and leads to reduction in their size. In addition to these effects, cigarette smoke including nicotine, influenced the trafficking of HSPC in vivo. Exposure of mice to tobacco smoke resulted in the inhibition of HSPC homing into the bone marrow.

Overall, this work is relevant to tobacco induced inflammatory disease. The studies suggest that constituents of tobacco smoke, especially nicotine, can influence the microenvironment of the bone marrow in addition to playing a proinflammatory role in target organs such as the lungs. Further the studies clearly indicate that nicotine can specifically target lung microvessels to induce the expression of vascular selectins and promote the selective recruitment of inflammatory leukocytes from the lung microcirculation. While we have accomplished majority of the goals proposed in the second specific aim, we will now examine the dynamics of how human leukocytes obtained from smokers interact within blood vessels under conditions of flow in vivo.

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B7

Gender differences in environmental tobacco smoke enhancement of the allergic response

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Epidemiological studies have shown that there is a genetic predisposition to allergy and that environmental factors such as second-hand smoke increase the phenotypic expression. These findings have stimulated further investigation on the sex distribution of children with allergic asthma from homes of smoking parents. While some studies have shown that male children exposed to environmental tobacco smoke (ETS) are more likely to be asthmatic than ETS-exposed female children; others have reported no sex differences in the prevalence of allergic asthma. These studies have prompted us to use our animal model of allergy to study gender differences in ETS and ambient air conditions. Previously we have shown that exposure of BALB/c mice to ETS enhances the allergic response to ovalbumin (OVA) in adult mice when compared with ambient air controls. To determine if gender predisposition is a factor in ETS enhancement of allergy to inhaled antigen we studied male and female mice exposed to immunogenic doses of OVA. Thus, 6 weeks old BALB/c mice, born and raised in either ETS or ambient air, were given 10 ug OVA in 2 mg of aluminiun hydroxide intraperitoneally followed by biweekly 20 minute exposures to a 1% aerosolized OVA. Quantification of the amount of OVA which was deposited in the lungs after a challenge of aerosolized OVA was demonstrated to be 1.7 ug OVA/mouse per exposure regardless of gender. However, assessment of the allergic responses revealed significantly ($p<0.05$) more OVA specific IgE and IgG1 in serum of female mice when compared to males. *In vitro* restimulation of the homogenized lungs revealed that the Th2 cytokines namely, IL4, IL5, IL10 and IL13 were elevated in both sexes but were significantly ($p<0.05$) higher in the females than in the males. To compare the allergic enhancement effect of ETS on females versus males a second experiment was conducted in which male and female BALB/c mice were born and housed in ETS and allergic responses were compared to those in ambient air. All mice exposed to ETS made enhanced levels of OVA-specific IgE, IgG1 and Th2 cytokines when compared to mice housed in ambient air. However, when we compared mice of the same gender, males were clearly more affected by ETS than females. Our findings showed that the most significant differences in allergic antibodies and Th2 cytokines between ETS and ambient air exposure groups occurred in males. These results are strongly indicative of a gender difference in exacerbation of the allergic response after exposure to ETS.

B8

Environmental tobacco smoke (ETS) significantly alters perinatal lung development in non-human primates.

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Early childhood development can be strongly influenced by exposure to a variety of factors including environmental tobacco smoke (ETS). The effects of ETS on the respiratory system are of particular interest since early exposure may enhance the rate of respiratory infection, cause decrements in lung function, and lead to an increased incidence and severity of asthma in young children. To determine the effects of exposure to ETS during perinatal development, we have developed a non-human primate model to expose Rhesus monkeys to aged and diluted sidestream cigarette smoke as a surrogate to ETS. Timed pregnant dams and their offspring were exposed to ETS from 50 days gestational age (DGA) to 75 days postnatal age (PNA) for six hours a day, five days a week. Exposure conditions were generated using a system that automatically smokes filtered research cigarettes (IR4F, University of Kentucky) using a standard puff volume of 35 cm for 2 seconds, once each minute. Smoke from the smoldering end of each cigarette was collected, aged and diluted prior to introduction into the exposure chamber system. Exposure conditions to ETS were characterized by an average (± S.D.) total suspended particulate (TSP) concentration of 0.99 ± 0.06 mg/m³, nicotine concentration of 257 ± 91mg/m³ and carbon monoxide concentration of 4.4 ± 0.5 ppm. These exposure conditions fall within a range that is environmentally relevant for children raised in homes where smoking occurs. The time of birth for each infant was in the expected range of 165 ± 10 DGA. At 75 days PNA each infant was examined to determine the proportion of immune effector and inflammatory cells in the lung air spaces, alterations in pulmonary and peripheral blood cytokine levels, and changes in the activity and distribution of pulmonary cytochrome P450 monooxygenases.

Exposure to ETS did not alter the number of cells recovered from the lungs by bronchoalveolar lavage. However, significant increases were noted in the proportion of monocytes (6.1 ± 0.3% in ETS vs. 2.1 ± 1.1% in controls), lymphocytes (1.0 ± 0.5% in ETS vs. 0.3 ± 0.2% in controls), and eosinophils (0.4 ± 0.1% in ETS vs. 0.1 ± 0.1% in controls). The level of IL-5, a cytokine involved in allergic and asthmatic-like conditions, was significantly increased to 158% of control in the lung tissues following exposure to ETS. Peripheral blood monocytes demonstrated a significant elevation in the level of IL-4 to 170% of control following exposure to ETS. Striking increases in microsomal cytochrome P4501A1 isozyme activity were observed in all lung subcompartments in infants exposed to ETS compared with controls. The highest levels of induction were found in the proximal airways (190-fold increase following ETS exposure compared with control) and parenchyma (168-fold increase following ETS exposure compared with control). The mid-level and respiratory airways were also induced 81- and 88-fold over control values, respectively. In comparison, previous experiments with rats exposed to an identical concentration of ETS demonstrated an increase in P4501A1 activity of only 2-fold above control. We conclude that exposure to ETS during perinatal lung development is associated with: (1) significant shifts in the proportion of inflammatory cells in the lungs, (2) significant increases the levels of pulmonary and systemic cytokines involved in the allergic response, and (3) significant elevations in pulmonary cytochrome P4501A1 in a site-specific manner in the neonate. These findings confirm that ETS exposure during perinatal development significantly affects the lungs of non-human primate infants.

B9

Strength training improves cardiac function in patients with chronic obstructive pulmonary disease

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The overall goal of this project is to improve effectiveness of medical treatment for patients with lung disease produced by cigarette smoking. Chronic obstructive pulmonary disease (COPD) affects approximately 14 million people in the United States. Exercise intolerance is a hallmark of this disease. It is becoming clear that these patients suffer not only from poorly functioning lungs but from poorly functioning muscles as well. We are focusing on two specific strategies to improve muscle function. First, in men the naturally occurring hormone, testosterone, is important in maintaining muscle mass; men with COPD have low levels of this hormone. We will determine whether administering testosterone increases muscle mass and exercise tolerance in COPD patients. Second, COPD patients are very sedentary; we will determine whether a vigorous program of strength training improves muscle function and exercise tolerance.

This study will randomize 48 men with COPD to one of four groups to receive: no exercise training and placebo injections, exercise training (progressive resistance training for major muscle groups of the lower extremity for one hour a day, three times a week) and placebo, no exercise training and testosterone injections (100mg per week), or both exercise training and testosterone. A large number of outcome measures are being assessed before and after the 10 week study period. Ultra-fast computerized tomography (UFCT) of the heart is one of these measures. This sophisticated scanning procedure allows detailed determination of both left and right ventricle dimensions and contractility. Patients are scanned both at rest and at the end of 4 minutes of steady state of cycle ergometer exercise at a work rate equal to 60% of the peak work rate tolerated in an incremental exercise test performed before the intervention.

UFCT scan data has been analyzed from pre- and post-intervention studies of 23 study participants. Though the study remains blinded as regards testosterone administration, we are able to distinguish between those who underwent strength training (n=12) and those who did not (n=11). These men averaged 68 years of age and FEV_1 averaged 40% predicted, signifying moderately severe disease; these characteristics did not differ between groups. Before the intervention, resting left ventricular ejection fraction (EF) averaged 57% and right ventricular EF averaged 40%. With exercise, average EF increased to 60% and 45%, respectively, with no significant differences between groups. After the intervention, resting left and right ventricular EF increased significantly in the strength training group (by 5.8% and 2.5%, respectively), but not in the non-training group (-2.9% and 0.6%, respectively). After the intervention, exercise left and right ventricular EF increased significantly in the strength training group (by 5.7% and 2.7%, respectively), but not in the non-training group (0.6% and 0.4%, respectively). These findings demonstrate for the first time that a rigorous program of strength training elicits an improvement in cardiac function in men with moderately severe COPD.

B10

Early cellular responses in diaphragm fibers of emphysematous hamsters following lung volume reduction surgery.

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With emphysema (EMP), the lungs become overinflated which impairs the function of the diaphragm, the most important of the breathing muscles. Over time, adaptations occurs in the diaphragm muscle in an attempt to preserve its ability to produce force. This occurs because the actual length of diaphragm fibers become absolutely shorter. Lung volume reduction surgery (LVRS) is a new procedure for patients with severe emphysema who, despite the best medical therapy available continue to have disabling shortness of breath. The procedure involves removing about a third of the most diseased portions of each lung. With the reduction in volume of the overinflated lungs, the shape and function of the diaphragm would be expected to be altered following this procedure. We hypothesize that in the acute postoperative phase following LVRS, rapid reduction in lung volume would result in diaphragm muscle fiber injury secondary to passive stretch imposed on the anatomically shortened diaphragm.

LVRS was performed 9 months following the induction of EMP via bilateral thoracotomies (chest wall incisions). Four groups of male hamsters were studied: 1) EMP/LVRS-D1 (1 day post surgery); 2) EMP/LVRS-D4; 3) EMP/sham surgery; and 4) Control (CTL)/sham surgery. Diaphragm fiber damage was determined by muscle perfusion with a low molecular weight dye (procion orange) to which the sarcolemma (muscle membrane) of normal fibers is impermeable and fibers identified by fluorescence microscopy. Fiber type proportions were determined histochemically. Maximum lung volume (MLV; at 25 cm H_2O pressure) was increased 1.6 fold with EMP compared to CTL animals. With LVRS, about 30% of each lung by wet weight was removed resulting in a 26% reduction in MLV. The percentage of diaphragm fibers with sarcolemmal injury was significantly greater in EMP/LVRS-D1 animals (10.9%) compared to the 3 other groups (0.4 to 1.6%). The proportions of injured diaphragm fibers were: Type I, 35%; type IIa, 46%; type IIx, 19%.

We conclude that acute passive diaphragm stretch following LVRS results in injury particularly in fibers likely recruited (used) for breathing efforts. We speculate that this early cellular response may be a necessary step leading to diaphragm remodeling following LVRS and could have important clinical implications in that diaphragm strength and ability to ventilate may be compromised in the short term.

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B11
Administration of insulin-like growth factor-1 (IGF-1) and corticosteroids in emphysematous hamsters: influences on diaphragm IGF-1.

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Patients with emphysema (EMP) are often treated with corticosteroids (anti-inflammatory drug) especially during acute exacerbations of the condition. A major side effect of corticosteroid treatment is skeletal muscle atrophy (i.e., muscle wasting) which results in weakness of the respiratory (breathing) muscles of which the diaphragm is the most important. The exact mechanisms by which muscle atrophy takes place are not completely known, but involve both a decrease in the rate of protein synthesis and an increase in the rate of protein degradation (proteolysis). The aim of this study was to evaluate the mechanisms whereby exogenous insulin-like growth factor-1 (IGF-1) prevent diaphragm fiber atrophy during corticosteroid administration to EMP hamsters.

Nine to 10 months after induction of EMP the animals were divided into 3 groups: 1) EMP only; 2) EMP + triamcinolone (dose: 0.4 mg/kg/day); and 3) EMP + triamcinolone + IGF-1 (IGF-1 dose: 600 mg/day by constant infusion). Drugs were provided over 4 weeks. Diaphragm fibers types were determined histochemically and fiber cross-sectional areas (i.e., fiber size) determined with a calibrated computer-based image analysis system. The intensity of IGF-1 staining (immunoreactivity) within single fibers was measured by microdensitometry. IGF-1 mRNA levels were determined by RT-PCR and IGF-1 peptide levels measured by a rodent specific radioimmunoassay. Lung volumes of EMP were increased (180 to 200%) compared to controls. Despite similar initial body weights, those of EMP + triamcinolone progressively decreased (-15%) during the study, while those of EMP and EMP + triamcinolone + IGF-I remained stable. Food intake was reduced to the same extent in both triamcinolone groups compared to EMP. Hypoglycemia (i.e., low serum glucose) was not observed with IGF-I infusion. Diaphragm weight was reduced with triamcinolone, but preserved with IGF-I administration. Diaphragm fibers proportions were similar among the groups. The cross-sectional areas of types I, IIa and IIx fibers were reduced (17 to 31%) with triamcinolone administration. By contrast, the concomitant provision of IGF-I prevented atrophy of all fiber types. Diaphram IGF-1 mRNA levels were similar across all groups. By contrast, the levels of IGF-1 peptide in the diaphragm were reduced (41%) in the EMP+T+IGF-1 rats. IGF-1 immunoreactivity confirmed this reduction in all diaphragm fibers.

We conclude that diaphragm fiber atrophy with T was prevented primarily by the hormonal influences of exogenously administered IGF-1 in that intramuscular IGF-1 expression was reduced possibly due to a negative feedback influence. We postulate that preservation of IGF-1 mRNA and peptide in the diaphragm with prolonged T administration was insufficient to offset the catabolic influences (i.e., negative influences on muscle protein turnover) of the corticosteroids. These results may have important clinical implications aimed at preserving respiratory muscle strength in patients with emphysema receiving large doses of corticosteroids during acute exacerbations or those requiring maintenance steroid therapy.

B12
Skeletal muscle structure and function in COPD

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The goal of this research is to determine if alterations in skeletal muscle structure and function contribute to exercise intolerance in patients with chronic obstructive pulmonary disease (COPD). While the predominant cause of exercise intolerance in COPD is the airflow obstruction, recent evidence suggests that the exercising skeletal muscle may also be impaired in patients with COPD. However, the prevalence of this skeletal muscle dysfunction and the mechanisms responsible for it are not well understood. By using a relatively small exercising muscle mass (knee extensor), the limitation created by the airflow obstruction in these patients can be removed and provides the means to accurately evaluate the limits to oxygen utilization in the exercising muscle of COPD patients. Using KE exercise, our results show that patients with COPD do demonstrate skeletal muscle dysfunction. At any given work level, oxygen (O_2) consumption is greater in COPD patients, without effecting maximal O_2 consumption, suggesting that skeletal muscle in COPD patients is less efficient in performing work than skeletal muscle from untrained controls.

One potential limitation to oxygen utilization by the working skeletal muscle is the number of blood vessels, known as capillaries, surrounding the muscle fibers. The more contact present between the muscle fibers and the capillaries, the greater potential for oxygen to get to the exercising muscles. In our patients, the results from skeletal muscle biopsies suggest that the skeletal muscle from COPD patients contains a greater percentage of Type II muscle fibers and a reduction in Slow Twitch Type I muscle fibers.

Vascular endothelial growth factor (VEGF) is a protein produced by the body, which promotes the growth of new capillaries. In normal exercising skeletal muscle, aerobic exercise is known to increase VEGF gene expression. Aerobic exercise training induces skeletal muscle capillary growth. Our results suggest that in untrained COPD patients, acute exercise produces a VEGF mRNA response 50% less than that of untrained control subjects, suggesting that the angiogenic response to exercise training may be impaired by COPD.

Finally, COPD patients are being exercise trained using single leg KE (on each leg individually) to test the hypothesis that COPD patients can improve the capillary-to-fiber interface in response to appropriate and sufficient training stimuli and improve O_2 consumption and conductance. In the single patient completed to date, there was no improvements in O_2 consumption or conductance during bicycle exercise, but exercise training resulted in a 25% increase in work rate and a 30% increase in leg blood flow during KE. In addition, the patient improved distance walked in a five minute walking exercise test.

Our data demonstrate that there are significant alterations in skeletal muscle structure and function in COPD. In addition, the results following single leg KE exercise training in a single patient suggest that inherent skeletal muscle function can be improved. However, during systemic exercise, the limitations imposed by the lung can not be overcome by exercise training. Future work will continue investigating the effects of exercise training on skeletal muscle structure and function in COPD.

Session C: California Multicultral Communities

C1

A new measure of acculturation for adolescent smoking prevention trials in multicultural populations

Unger, Jennifer B., Gallaher, P., Ritt-Olson, A., Palmer, P.H., Johnson, C.A.
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Previous studies have shown that acculturation to the United States culture is a risk factor for adolescent smoking in several ethnic minority groups. Brief, valid measures of adolescent acculturation are necessary for population-based research on acculturation and adolescent smoking. However, most available acculturation surveys are not appropriate for adolescent health surveys because they are too long and complex, are not applicable to multiple ethnic groups, or are purely language-based.

This study developed and validated a brief, multidimensional, multicultural scale of adolescent acculturation for use in smoking prevention research. A sample of 317 6th-grade students in 13 multicultural schools in urban Los Angeles completed the new scale and several other established measures of acculturation. The new acculturation scale generates four subscores: U.S. Orientation, Other Country Orientation, Biculturalism, and Marginalization. The U.S. Orientation and Biculturalism scales were significantly correlated with other measures of acculturation: the ARSMA-II, English language usage, and generation in the U.S. These correlations with established acculturation measures support the validity of the new measure. Item response analyses revealed that items concerning use of U.S. media were best at discriminating between low and moderate levels of acculturation, while items concerning customs at home and feeling comfortable with people from the U.S. were best at discriminating between moderate and high levels of acculturation. Preliminary analyses indicated that the U.S. Orientation scale was associated with a higher risk of lifetime smoking and susceptibility to smoking among Asian American and Pacific Islander adolescents, although it was associated with a lower risk of lifetime smoking among Latino adolescents. After we have collected baseline data from our full longitudinal sample, we will conduct additional analyses of the associations between acculturation and adolescent smoking to gain a more complete understanding of these associations.

This work is relevant to the prevention of smoking among adolescents in a multicultural population. It is essential to understand the role of acculturation in increasing or decreasing the risk of adolescent smoking. This information can be useful in the development of culturally relevant smoking prevention programs for adolescents. The creation and validation of an appropriate acculturation measure is an important first step toward this goal.

C2

Acculturation, depression and nicotine dependence: A preliminary analysis

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A close relationship has been found between smoking and depression in the general population. The lifetime prevalence rate of major depression among smokers is almost 3 times higher than among non-smokers. Little is known empirically whether the relationships between smoking and depression are similar or different among smokers who are not or less acculturated to the American culture. The preliminary analysis was based on 44 participants recruited for Project CHAMPS, a study that will recruit 400 current smokers from the Chinese and non-Chinese populations using a longitudinal survey design to examine the associations among depression, readiness to quit smoking, and smoking behaviors.

Project CHAMPS was described as a study to examine how culture, health and mood influence the processes of smoking. Results were based on data collected from 44 participants with 50% female, mean age 40.7 years and 25% foreign-born who completed baseline assessment between July 25, 2000 and Oct 14, 2000. The participants included 24 (54.5%) Caucasian, 10 (22.7%) African American, 5 (11.4%) Chinese, 2 (5%) Native American and 3 (6.4%) of other ethnicities. Among the 11 participants who were born outside of U.S., 9 (82%) began smoking regularly prior to their immigration to the U.S. Participants smoked at least 5 cigarettes during the past 7 days prior to the baseline assessment, with the average daily smoking rate of 13.4 cigarettes. Using the Composite International Diagnostic Interview (CIDI), 30 (68.2%) met the DSM IV diagnostic criteria for nicotine dependence and 16 (36.4%) participants reported a lifetime history of major depressive episodes.

Foreign-born participants scored significantly lower on self-rated proficiency in both spoken and written English, and in the total score of the General Ethnicity Questionnaire measuring the degree of acculturation to the American culture across several dimensions. American-born smokers reported a significantly higher current level of depressive symptoms (17.8 vs. 6.3), as measured by the Center of Epidemiological Studies–Depression Scale (CES-D), when compared to those who were foreign born. However, both American-born and foreign-born participants were statistically similar in terms of rates of daily smoking (15.6 vs. 8.6), nicotine dependence (66.7% vs. 72.7%) and history of major depression (42.4% vs. 18.2%). No statistical significant relationships were observed among depression and nicotine dependence measures with other measures of acculturation.

The preliminary results suggested a potential impact of acculturation in smoking behaviors and depressive symptoms reported among smokers. The results are not conclusive due to the small sample size. Recruitment is on-going and these results will be re-analyzed based on more data that will be available at the time of the presentation.

POSTER SESSIONS
Session C: California Multicultral Communities

C3

Healthy generations: a study of culturally-specific parental prompts to smoke among youth, results from middle school student surveys.

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San Diego State University

Our previous work with Latino youth indicated that Latino parents, more so than non-Latinos, may prompt their children to engage in behaviors putting them in direct contact with cigarettes or inadvertently encourage them to "practice" smoking-related behaviors. As part of a larger prospective study examining these prompts as smoking initiation risk factors, this study documented the extent of parental prompts. In this study, prompting is defined as seven parent behaviors, including asking the child to: (a) empty/clean ashtrays, (b) bring cigarettes to parent, (c) receive tobacco industry promotional gear, (d) buy cigarettes for parent, (d) light parent's cigarette with a match or lighter, (e) start the cigarette in his/her own mouth and then pass it to parent, and (f) smoke with the parent.

A total of 10,500 recruitment letters and consent forms, in both English and Spanish, were distributed to all 7th and 8th grade students in the Sweetwater school district. Parents returned 5908 (56%) signed consent forms with 4228 (72%) giving permission for their child to participate in the study. A total of 3624 (86%) middle school students completed surveys. The poster will present findings from the child surveys including prevalence rates of ever and past 30-day smoking in the Sweetwater district and discuss other findings indicating that:

1. Parental prompting was predictive of children's smoking independently of parental smoking.
2. Parental prompting rates were similar for Latino and non-Latino youth, and parental prompting was associated with a higher prevalence of youth smoking regardless of ethnicity.
3. Four of the seven prompts included in the analysis were predictive of youth smoking (bring cigarettes, light cigarettes for parent, light in own mouth, and smoke with parents).
4. A higher familism score was significantly associated with a lower risk of past-month smoking, regardless of ethnicity.

C4

Does change in parents' attitudes towards smoking predict adolescents' smoking across different ethnic groups?

Ellickson, Phyllis L.
RAND

The health risks associated with cigarette smoking are numerous and well documented. While adult smoking has declined, decreases in youth smoking have lagged, and the age of smoking onset has actually decreased.

Ethnicity has been identified as a predictor of adolescent smoking and there are ethnic differences in rates of smoking. Generally, white and Hispanic youth have been found to smoke more than their African American and Asian counterparts. In addition, there are consistent ethnic differences in the factors that are associated with adolescent smoking.

We explored predictors of adolescent smoking across four ethnic groups – White, Hispanic, Asian and African American. A review of literature on cultural differences in parenting and socialization revealed that African American parents emphasize obedience and social conformity, Asian parents emphasize self-control and not shaming ones family, white parents emphasize individual freedom and independence while Hispanic parents emphasize permissive acceptance. This led us to make the following predictions:

- African American and Asian parents disapprove of smoking more than white and Hispanic parents.
- This difference would predict rates of smoking with African American and Asian youth smoking less than white and Hispanic youth.
- Declines in parent disapproval of adolescent smoking would be associated with increased smoking across ethnic groups.

Methods and Results:
Survey data on a wide range of issues related to substance use were collected from 4390 7th grade students. Similar data were collected when students were in the 12th grade. 71% were white, 8% were African American, 9% were Latino and 9% were Asian.

We predicted heavy smoking in the 12th grade, defined as smoking one or more cigarettes per day in the past month. Heavy smoking was rare in the 7th grade (ranging from .6-4% across ethnic groups), BUT far more common by grade 12 (ranging from 4.3-22.4%). Concurrently, parent disapproval rates decreased, but they did so more for white and Hispanic youth (who were also the heaviest smokers in grade 12).

Univariate regression models, conducted for each ethnic group, revealed that adolescents' perceptions of change in their parents' attitudes towards smoking predicted smoking for all four groups. Expanded regression models also accounted for demographic characteristics; problem behaviors; attitudes towards smoking; family and school variables and environmental exposure to smoking and to attitudes towards smoking. The only variable that predicted heavy smoking in the 12th grade across all four ethnic groups was change in parents' attitudes towards smoking. Ethnic differences in the other predictors were observed with more significant predictors for white youth than for any of the other groups.

Conclusions: Results from this study point to decreased parental disapproval of smoking during the adolescent years as a key predictor of heavy smoking among adolescents and an important variable in explaining ethnic differences in heavy smoking among older adolescents. One implication is that prevention efforts should help parents effectively communicate their disapproval of smoking throughout the adolescent years.

C5

Recruitment and data collection issues in tobacco research with African American families

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According to recent data from the Centers for Disease Control, smoking among teenagers was on the decline until the early 1990s, when the rate of smoking abruptly increased. Although rates for African American female teenagers continue to decline and the overall smoking rate for African American youth is lower than those rates for white and Latino youths, cigarette smoking has nearly doubled among African American male teenagers since the early 1990s. The reasons for these striking changes are unexplained at this time. The purpose of the current study is to examine characteristics of African American youth and "African American youth culture" that positively and negatively predict cigarette smoking initiation and continued use.

The study focuses on African American 10-12 year olds who live in the San Francisco Bay Area. In order to develop an age-appropriate, culturally relevant questionnaire, six focus groups were conducted with 38 10-12 year olds. The resultant child questionnaire includes questions about the child's thoughts toward smoking, school, friends, their expectations towards smoking, their knowledge on the health effects of smoking and their use of other substances such as alcoholic beverages and marijuana. In addition to completing the questionnaire, the child is weighed and measured to calculate body mass index and provides a saliva sample to measure cotinine levels as a validity check. The adult questionnaire consists of questions that include the following topics: future expectations of child, parents' attitudes and behaviors regarding smoking and alcohol use, and demographic information.

Recruitment of the cohort started with the help of community organizations and community events that provide services and information to African American families. Two hundred families have been recruited from San Francisco, Oakland, Richmond, and San Mateo County. Twenty-five percent of the families completed interviews and 15% were ineligible. A significant amount of effort has focused on overcoming barriers to recruitment and data collection. These barriers include handling issues of potential participants' mistrust of research, parents' concerns about home-based interviews, difference in children's versus parents' level of enthusiasm to participate, interviewers' safety issues, and participants' cancellations of scheduled interviews. Current recruitment efforts target enlisting the assistance of principals and teachers within local school districts.

C6

Reducing minors= access to tobacco: Project CHALK

Landrine, Hope
Public Health Foundation

The purpose of this innovative, 3-year, community-intervention project was to reduce sales of cigarettes to youth. The project is innovative in the sense that we did not focus on the store clerks who sell cigarettes to minors, but instead, focused on the community of adults who observe such sales yet say and do nothing to stop them. Our goal was to motivate adults in the communities surrounding the stores to object when they observe sales of tobacco to youth, and to shop only in stores that do not do so. This intervention was conducted, not by researchers, but by parents and adults from the community.

In Year 1, a random set of 72 small stores was identified to be the target of the intervention, with 24 stores each in Black, White, and Latino communities. Black, White, and Latino youth then attempted to purchase cigarettes in each of the 72 stores; these initial purchases established the baseline rate of sales of tobacco to youth prior to intervention. The 72 stores then were randomly assigned to three intervention groups (Wave 1, Wave 2, and Wave 3). For Year 2 and Year 3, the intervention was conducted (using a multiple-baseline design) first in the Wave 1, then in the Wave 2, and finally in the Wave 3 stores, with the rate of sales of tobacco to youth assessed immediately after each intervention, and again 6 months after the end of the project.

Results revealed that sales to youth in the Wave 1 stores decreased from 10.4% at Baseline to 6.2% at final follow-up; in the Wave 2 stores, sales decreased from 16% at Baseline to 6.9% at final follow-up; and in the Wave 3 stores, sales decreased from 12% at Baseline to 4.6% at final follow-up. The decreases in sales in the Wave 1 and 2 stores did not occur immediately after the intervention however, but instead, later, suggesting a delayed intervention effect or a possible spill-over effect from intervening in neighboring stores. The intervention was most clearly successful in the Wave 3 stores, where sales decreased immediately after the intervention, and then remained low through final follow-up. Hence, only a portion of the decreases in sales of tobacco to youth can be attributed to the intervention.

These findings tentatively suggest that changing community attitudes and behaviors might be added to current merchant-education efforts to maximize the success of efforts to reduce youth access to tobacco.

POSTER SESSIONS
Session C: California Multicultral Communities

C7
Explaining racial differences in smoking

Landrine, Hope
San Diego State University

The purpose of this 2-year project was to examine variables that might explain recalcitrant, Black-White disparities in smoking. In addition to investigating the well-known effect of socioeconomic status (SES), two unique variables were examined: racial discrimination and racial segregation. The specific aim was to assess the extent to which the latter two variables account for racial differences smoking prevalence, quit rates, age of smoking initiation, stage of readiness to quit smoking, and degree of nicotine addiction.

This cross-sectional study entailed a survey mailed to a random sample of 4,000 Black and 2,000 White California adults ages 18 to 73 years who were residents of five specific Metropolitan Statistical Areas that differ in their degree of racial segregation. The survey return rate was 35% (N = 2,116). Preliminary findings thus far revealed that both lifetime (entire life) and recent (past year) racial discrimination are significant predictors of smoking among Blacks, and specifically, significantly increase Blacks' risk of early initiation of smoking, current smoking, and smoking even when physically ill. Analyses of segregation effects, segregation X discrimination effects, and racial comparisons are currently underway. Nonetheless, our findings to date suggest that racial discrimination plays an important role in Black smoking, and so imply that addressing less self-destructive ways of coping with racism may need to be added to culturally-tailored smoking prevention and cessation programs to improve their effectiveness with Blacks, and thereby reduce the high cost of tobacco use in the state.

C8
Hmong cultural practices and patterns of tobacco use

Krenz, Vickie / Lee, Justin
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Tobacco use is the single most preventable cause of death and disease in the United States with an estimated 418,000 premature deaths each year. Among Asian American/Pacific Islanders, there are an estimated 12,000 premature deaths each year. Cigarette smoking and tobacco use among Asian/Pacific Islanders are varied, ranging from 43% among Southeast Asians to 28% for Chinese. Studies that have targeted Southeast Asians have focused primarily on Cambodian, Laotian, and Vietnamese. However, there are no studies that estimate smoking prevalence among the Hmong, nor research that explores the tobacco patterns and practices of this unique group. This project focused on Hmong cultural patterns and practices of tobacco use.

Because Hmong cultural tobacco practices and patterns have not been researched, this project focused on gathering preliminary information to bolster the core understandings of Hmong tobacco use. The information was used to develop a survey tool, to be applied in a future research project, that will enable the full exploration of smoking patterns of this population with a valid and reliable tool. The specific aims of the project were to gather information from the Hmong population in California re: 1) The perceptions Hmong hold toward tobacco, 2) the nature of current tobacco use in the Hmong population, 3) the cultural factors that influence and reinforce tobacco use, 4) how length of residence in the U.S. affects tobacco use, and 5) what processes will lead to health-related behavior change in the Hmong.

Information was gathered through six culturally appropriate focus groups that were conducted with ten to twelve individuals in each group. Separate focus groups were conducted for: (1) Males 30-55 years, (2) females 30-45 years, (3) males 19-29 years, (4) females 19-29 years, and combined male/female groups for those 15-18 years (5) and 10-14 years (6). Each focus group was guided in their discussion by a Hmong facilitator exploring key issues in the Hmong language about tobacco-related practices and patterns. Information gathered was analyzed and categorized to capture overarching themes emerging from the focus group responses.

Based on the information obtained from the first round of participant focus groups, a second set of focus groups met to further refine the identified themes and issues. Additionally, the focus group participants were given the initial draft survey to assess the appropriateness of the content and constructs of the tool, which has been finalized and will be administered in a future research effort. The second round of six focus groups were comprised of the same age/gender groups as in the first round, but involved different individual participants recruited from throughout the community. The results of the project produced (1) a preliminary understanding of the tobacco-related patterns and cultural practices of the Hmong, and (2) an initial survey instrument to further assess smoking and tobacco use among a cultural group for which a paucity of research exists.

C9

Lowering smoking & ETS risks in immigrant Pacific Rim youth

Johnson, C. Anderson; Unger, J.B.; Gallaher, P.; Palmer, P.H.; Baezconde-Garbanati, L.; Lichtman, K.; Chou, C.; Trinidad, D.; Ritt-Olson, A.; Andrion, J.B.
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Immigration from Pacific Rim countries is robust. California's highest and lowest smoking rates for youth occur among immigrant Pacific Rim populations. Research has elucidated the influence that immigrant Asian and Hispanic/Latino parents typically have over their children. However, little is known about how their traditional cultural norms and parenting styles either promote or deter smoking among their children. Also unclear is the extent to which smoking behavior among Asian and Hispanic/Latino youth is affected by the clash between American culture and the traditional culture of their parents. Our objectives are to develop a culturally attuned smoking prevention program for Asian and Hispanic/Latino middle-school children, the age group at highest risk for smoking initiation, and to assess program effectiveness. To this end, we will identify how familial, cultural, and environmental influences affect the social and individual factors germane to smoking prevention in our targeted populations.

Over the past year, we have focused on the development and pilot testing of surveys and a multicultural smoking prevention curriculum. The survey assesses ethnicity, SES, academic achievement, family structure, smoking behavior, acculturation and cultural values, connectedness to parents, peer influences, tobacco-related media exposure, personality/psychopathology, and health-related values. Four waves of the survey were administered to first-year students in 22 public (N=2,507) and 25 private (N=492) middle schools. The average age of respondents was 11.5 years old (SD = .58 years); 46.3% were male and 53.7% female. The ethnic distribution of the total sample was 47.1% Hispanic/Latino, 27.2% Asian/Pacific Islander, 6.0% Filipino, 13.2% White, 3.9% African American, and 2.5% Other. The estimate of lifetime smoking (ever smoked, even a few puffs) was 11.8%. Smoking rates varied by ethnicity and for Hispanic/Latinos was 13.8%, Asian/Pacific Islanders, 6.7%, White non-Hispanics, 11.8%, and Filipinos, 15.7%. The rate for Hispanic/Latinos exceeded that of non-Hispanic Whites, a result that replicated in each of the pilot studies. This indicates a shift as reported in earlier studies, where smoking rates were highest for non-Hispanic Whites. Early results suggest that cultural values, including Filial Piety (a child's duty to respect parents) and Simpatia (importance of smooth, harmonious social relations) may be protective against smoking.

Pilot surveys, student focus groups and interviews, ethnographic studies, and cultural experts, have provided our curriculum writers with input for the development of a multicultural smoking prevention curriculum, stressing the cultural values of both Hispanic/Latinos and Asians. Influenced heavily by social normative theory, the seven-lesson curriculum considers elements of attribution theory, acculturative stress, social networks, self-efficacy, and family relationships, as well as features of culture.

By 2025 children of Hispanic and Asian families will comprise 77% of the California school population. Given the increasing number of Pacific Rim immigrants and vulnerability of middle-school children to the initiation of tobacco use, this research is relevant to the understanding and prevention of smoking in California's youth.

C10

Ethnic variation in peer influences on adolescent smoking and susceptibility

Unger, Jennifer B.; Rohrbach, L.A.; Cruz, T.B.; Baezconde-Garbanati, L.; Howard, K.A.; Palmer, P.H.; Johnson, C.A.
University of Southern California

Previous research has indicated that the influence of peers on adolescent smoking may differ across ethnic groups. Although many studies have focused on African Americans, Hispanics, and Whites, few studies have included Asian Americans, Pacific Islanders, and Multi-ethnic adolescents as distinct groups. Using data from a statewide sample of 5870 8th-grade adolescents in California, this study examined ethnic differences in the association between peer influence variables and smoking behavior and susceptibility. Informational peer influence (best friends' smoking behavior) and normative peer influence (prevalence estimates of peer smoking) were investigated. We hypothesized that informational peer influences would be stronger among whites (whose families originate primarily from the individualistic cultures of the U.S. and Western Europe) than among Asian Americans, Pacific Islanders, Hispanics, and African Americans (whose families originate primarily from collectivist cultures). Conversely, we hypothesized that normative peer influences would be stronger among ethnic minority adolescents from collectivist cultural backgrounds than among whites.

Consistent with previous studies, friends' smoking and prevalence estimates of peer smoking were risk factors for past 30-day smoking and susceptibility to smoking across ethnic groups. The influence of friends' smoking behavior was stronger among whites than among several other groups: Pacific Islanders, African Americans, and Hispanic/Latinos. The influence of prevalence estimates of peer smoking was stronger among Whites than among Multi-ethnic adolescents. These results indicate that cultural factors may play a role in peer influences on smoking initiation. Smoking prevention interventions for adolescents should address the differences in peer influences across ethnic groups.

These results are relevant to the prevention of adolescent smoking, which ultimately may reduce the burden of tobacco-related disease. These results provide new information about the ways in which cultural and social influences interact to encourage or discourage smoking among adolescents. This knowledge can be used to develop more effective smoking prevention curricula for adolescents in a multicultural population.

D1

Establishment of a cohort of California twins for the study of tobacco-related diseases

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The California Twin Program (CTP) consists of a population-based cohort of twins born in California who have completed a questionnaire that obtains information on many lifetime exposures including smoking and exposure to environmental tobacco smoke. Details of the development of the cohort are presented here.

The record of all multiple births in the State of California occurring between the years 1908 and 1972 (200,590 individuals) has been linked on 3 occasions since 1991 to the records of the Department of Motor Vehicles (DMV) to obtain addresses for California-born twins (112,468 successes). Over the past 10 years, we have sent introductory letters to 'waves' of twins so identified (and another 8,214 not found by DMV linkage, identified by their co-twins), starting in 1991 with those over the age of 37. After removing twins from our mailing lists whose introductory letter was returned with a bad address, we mailed a 22-page questionnaire to the remainder (102,258). To date we have had responses from 41,367 individuals. Future DMV linkages will provide the opportunity to increase this number. We are in the process of linking the entire cohort to records of the California Cancer Registry and the Los Angeles Cancer Surveillance Program to identify, among others, cases of tobacco-related cancers.

We present analyses of response rates among interesting subgroups, and the representativeness of the current respondents in comparison with the California-born 1990 resident population (obtained from the 1990 census), and in comparison with the original roster of twins.

We conducted a small sub-study that indicated that as many as 20% of non-respondents may never have received the questionnaire because we did not have their correct address. This allowed us to calculate overall response rates as follows: 1) presuming that all people we sent a questionnaire received it (response rate=40.4%) 2) removing those people we know did not receive it (deceased, Post Office returns: response rate=42.2%) 3) estimating the number of non-respondents who did not receive the questionnaire (response rate=52.7%).

The success of the DMV linkage was better for males than for females, and better for older than younger twins. Post office returns were higher among younger males than other age/gender groups. Overall, the response rate was highest among young females, and lowest among older males.

The cohort of twins born 1908-72 did not differ from the California-born 1990 resident population (aged 22-82) in terms of gender, race or education. The respondents were slightly more poorly educated, slightly more likely to be female, far more likely to be white (than Hispanic, Black, or Asian).

We assess the utility of this cohort for providing a source of study population for three types of studies of tobacco-related disease 1) 'prevalence' studies using data already collected from the 41,367 twins as though they were a population-based group of Californians (eg documenting the role of parental smoking in smoking rates of their children); 2) studies comparing exposures and diseases between twins in the same pair (eg parental smoking in twin pairs discordant for asthma), and 3) studies using the identified twins for further investigation and sampling (eg 7RT-0134H also presented at this meeting).

D2

The effect of cigarette smoke on IL-4 and IL-5 levels in identical twins

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Cytokines interleukin-4 (IL-4), interleukin-5 (IL-5 and interleukin-13 (IL-13) are involved in the allergenic response and appear to be elevated in asthmatics. One previous study reported that current smokers have higher IL-4 and IL-5 levels compared to non-smokers, but this study used convenience samples and did not control for gender, age or ethnicity. We have previously shown that twins have similar levels of certain types of cytokines and other studies have demonstrated strong correlation of IgE (a strong mediator of the asthmatic response which is increased by IL-4 and IL-13) in identical twins. The goal of this research project is to determine the effect of tobacco smoke on cytokines related to asthma, principally IL-4, IL-5 and IL-13, by comparing these levels in healthy identical twins who have different levels of tobacco smoke exposure. The advantage of this study design is that, by using identical twins, we can hold constant other factors that may influence cytokine production and response to tobacco smoke such as genetics and early childhood exposure to tobacco or other allergens. The twins were obtained using the California Twin Program, a population-based resource for studying tobacco-related diseases funded by the TRDRP (8RT-0107H).

To date, 164 (75%) out of 218 pairs successfully contacted agreed to participate. Of these, 81(49%) pairs were eligible: 13 pairs were discordant for ever/never smoking (one twin currently smokes, the other never did), 25 pairs were discordant for quitting (one twin currently smokes, the other used to and quit), 50 pairs were concordant for smoking but at different levels (both smoke but one twin smokes more), and 5 pairs were discordant for passive smoke exposure (neither twin smokes, but one twin lives with a smoker and the other does not).

Preliminary laboratory results on a small sample of 17 twin pairs (34 twins) show that both IL-4 and IL-5 levels are consistently higher in the twin who smokes compared to his/her twin that doesn't (mean enhanced IL-4 in smokers vs. non-smokers = 6.33 vs. 3.76 pg/ml, $p < 0.05$; mean IL-5 levels in smokers vs. non-smokers = 1.57 vs. 8.93 pg/ml, $p < 0.01$). Among twins who both smoke, levels of both cytokines were higher in the twin that smoked more although the dose-response trend was not significant due to small numbers. We plan to perform laboratory tests on the 126 samples (63 pairs) obtained to date and present the results for IL-4 and IL-5 levels by smoking status in this expanded sample.

This study will increase the understanding of how tobacco smoke leads to the development of asthma, or worsens existing asthma, and may help us to design effective interventions. We will use the results of this study to develop a proposal to examine the interaction between tobacco smoke, household allergens and cytokine genes as risk factors for childhood and adult onset asthma.

POSTER SESSIONS
Session D: Epidemiology

D3

Complex segregation analysis of smoking in families: Evidence for a genetic mode of transmission in nuclear families with a high prevalence of smoking

Swan, Gary E.[1] (In collaboration with L. Cheng[2]; D. Carmelli[1]; K. Hudmon[1]; K. Hemberger[1]; H. Hops[3]; J. Andrews[3]; L. Tildesley[3]; D. McBride[3])
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Numerous studies of twins support the hypothesis that cigarette smoking is influenced by both genetic and environmental factors. A growing number of studies based on the case-control design have identified a higher prevalence of certain variants of candidate genetic markers in smokers than in nonsmokers. While findings from both the twin and candidate gene literature are supportive of the hypothesis that smoking is in part genetically determined, few studies of smoking in families have described the pattern of transmission (genetic or otherwise) from parents to children. This study is designed to fill these research needs.

The work presented in this poster is from a component of the TRDRP-sponsored Integrated Research Project (IRP), entitled *Genetic and environmental influences on tobacco use in adolescents*. It is being conducted in collaboration with investigators from SRI International (G. Swan, D. Carmelli, K. Hudmon), the Oregon Research Institute (H. Hops, J. Andrews, L. Tildesley), UC San Francisco (N. Benowitz, K. Wilhemsen), and Stanford University (B. Koenig, L. Caron).

Objectives of the IRP include: 1) the collection of family histories of smoking, and 2) analysis of these data to examine the familial pattern of transmission of smoking. Four hundred sixty eight family smoking histories of all first-degree biological relatives of index cases were collected primarily by telephone interview. Index cases are participants in a longitudinal study, now in its 15th year of follow-up, of psychosocial and behavioral predictors of substance use. The smoking-related phenotype in this report is eversmoking (defined as having smoked 100 or more lifetime cigarettes and determined for all relatives from the report of index cases). We applied complex segregation analysis to test whether eversmoking aggregates in families and, if so, to further test whether there is evidence for a pattern of genetic transmission. Analyses were conducted using SAGE. We used the maximum likelihood ratio test to determine the most parsimonious model. Using the full data set, while we found evidence for familial aggregation, evidence for genetic transmission was inconclusive, with a general transmission model providing a better fit to the data than a genetic transmission model. Because smoking behavior is a complex trait at both the phenotypic and genotypic levels, we then focused our efforts on data from families with a high prevalence of smoking (3 or more first-degree relatives identified as eversmokers). Utilizing family smoking history data from 128 families with three or more smokers for whom we had complete data, we found that the Mendelian transmission model was not rejected ($c^2 = 3.27$, df = 3, $p > 0.05$), while the environmental transmission model was rejected ($c^2 = 8.06$, df = 3, $p < 0.05$), suggesting the presence of a segregating genetic substrate in these families. The best fitting model included terms for a dominant genetic effect along with a positive spousal correlation and a negative maternal correlation, consistent with our recently published findings in families from a general population study. These results suggest that while strict genetic transmission of eversmoking is not evident across all families, evidence consistent with Mendelian transmission is present in families with a high prevalence of eversmoking. This finding highlights the utility of focusing genetic investigations of smoking on "high-risk" families ostensibly because of less heterogeneity in the underlying biological and environmental substrate.

D4

Smoking during pregnancy: chromosome Damage in mothers and newborns

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The goal of this research is to evaluate the effect of maternal cigarette smoking during pregnancy on both the mother and newborn. It is of particular interest to understand how smoking and inherent genetic susceptibility relate to observed chromosomal aberrations in the circulating blood. Approximately one quarter of the population is inherently susceptible to chromosomal damage while 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 three hundred mothers and their newborns are being collected for this study. Peripheral blood lymphocytes from the mother and the fetal side of the placenta are 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 scored from each maternal and newborn sample. The alterations are quantified using PAINT, a cytogenetic approach 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, which request information about smoking history, maternal blood samples are being evaluated for cotinine levels and 4-aminobiphenyl-hemoglobin (4-ABP-Hb) adducts, two biochemical measures of tobacco exposure. This biochemical information will reduce the risk of potential recall bias for self-reported tobacco use. Comparison of preliminary biochemical data to maternal self-reports suggest good correlation between baseline cotinine and 4-ABP-Hb adduct levels and maternal self-reported cigarette smoking prior to and early in pregnancy.

To date, 162 blood samples have been collected from Caucasian mother/newborn pairs, 57 of which have been scored for chromosome aberrations. Fifty-six African-American sample pairs have been collected and 33 scored. Sociodemographic data have been collected from all patients, a subset of which was currently available. Chromosome aberration data and smoking status information was available for 12 mothers and 15 newborns. Preliminary analyses evaluated the effect of maternal smoking during pregnancy, ever smoking, maternal age, and race on chromosomal aberration frequency. Neither mother's age nor race were significantly associated with chromosome aberration frequencies in this population. The association between smoking status and chromosome aberration frequencies in the maternal population was marginally suggestive of significance (P=0.10). In a regression analysis evaluating the effect of all variables on the chromosomal aberrations in newborns, ever smoking correlated significantly with DNA damage (P=0.03).

Future objectives include concluding sample collection, scoring samples for chromosome aberrations, and determining genetic susceptibility to DNA damage. We will determine if 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, to the mother and newborn, are critical for the improvement of the health of the individual and the community. This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract No. W-7405-ENG-48.

D5

Maternal smoking and congenital abnormalities: results from the California Twin Program

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The California Twin Program (CTP) consists of a population-based cohort of twins born in California who have completed a questionnaire which obtains information on many lifetime exposures including smoking and exposure to environmental tobacco smoke. Details of the development of the CTP are presented under 8RT-0107H.

The effect of maternal smoking on the developing fetus has been previously shown to increase the likelihood of a low birth weight infant; however there have been few studies that have assessed the effect smoking on the development of congenital abnormalities. In the CTP the following congenital abnormalities have been studied in relation to maternal (and paternal) smoking: clubfoot, congenital heart problem, cerebral palsy, muscular dystrophy, cleftlip, deafness, Down's syndrome, mental retardation, spina bifida, strabismus, and other congenital problems. Based on data from over 21,000 twin pairs we have found elevated unadjusted risk ratios for maternal smoking and clubfoot (1.61, p=.06), spina bifida (2.25, p=.04), and strabismus (1.87, p=.001). Data from additional pairs will be included in the final analysis.

Additional information on the occurrence of these problems by type of twin pair, educational attainment of the parents, age of mother and the combination of effects from maternal and paternal smoking will be presented. Estimates of the expected number of these abnormalities vs. the observed number will be made and issues related to ascertainment of these conditions will be discussed. These findings add to the many reasons why mothers should refrain from smoking during pregnancy.

D6

Oral clefts, maternal smoking and genetic variation of glutathione-S-transferases

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Orofacial cleft malformations are among the more common congenital malformations with a reported prevalence between 1 and 2 per 1,000 live births. Although largely unknown, their etiology is suspected to be heterogeneous. In some studies however, smoking during pregnancy has been identified as a risk factor. Recently we found that about 24% of pregnant California women smoke during pregnancy. We previously identified a small increased risk for clefts among mothers who smoked more than a pack per day. In an effort to better understand the increased risk of oral cleft malformations when the mother smokes during pregnancy, we are genotyping fetal-derived DNA samples for known polymorphisms affecting the activity of glutathione-S-transferase enzymes. Glutathione-S-transferases are enzymes involved in the detoxification of chemicals such as the toxins found in tobacco smoke.

Blinded archival dried blood spots corresponding to an existing epidemiological data set of known cases of oral facial clefts (cleft lip or cleft palate) as well as unaffected controls were obtained for these studies. Following genomic DNA extraction of 423 cases and 294 non-malformed controls, genotypes for glutathione-S-transferase M1 (GSTM1) and T1 (GSTT1) were determined by PCR amplification followed by restriction fragment length polymorphism (RFLP) analysis. The variant form (null) of each of these GSTs is a major deletion encompassing several exons of each gene. Infants who are homozygous null have complete absence of any functional enzyme.

The resulting genotypes are then paired with epidemiological data previously collected through interview with the mothers concerning behavioral risk factors such as smoking prior to and/or during pregnancy. Compared to non-smoking mothers whose infants had at least one functional copy of the GSTM1 gene, we found an increased risk (odds ratio = 1.6) for an oral cleft among smoking mothers whose infants were homozygous null. For GSTT1, we found an even higher risk. Smoking mothers whose infants were homozygous null for GSTT1 had an odds ratio of 2.7 for oral cleft compared to the reference group.

These results show significant interactions between maternal smoking and the developing embryo's inability to detoxify chemicals via the GST pathway. This is one of the first examples of a gene-environment interaction as the cause of a common structural malformation in humans.

POSTER SESSIONS
Session D: Epidemiology

D7

Tobacco smoke exposure and urinary hormones

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Tobacco smoke contains suspected reproductive toxicants that may act by affecting hormone production or metabolism. In a previous analysis, we found that women who smoked heavily had shorter and more variable menstrual cycles on average than non-smokers. We measured urinary metabolites of estrogen and progesterone, the ovarian steroids, in the same data set. In preliminary work, we have noted some changes in the excretion pattern of these metabolites among smokers. The goal of the current project is to measure a pituitary hormone, follicle stimulating hormone (FSH), to aid in determining the mechanism of effect of tobacco on this endocrine system. FSH is critical to the growth and development of follicles prior to ovulation and has been proposed as a marker of ovarian competence.

The data was originally collected in a prospective study of about 400 women who saved first morning urine samples for up to 6 months, or until pregnancy. Steroid metabolites were measured daily and used to define various menstrual and hormonal characteristics of the more than 1500 cycles completed. The current project funds measurement of FSH in about 300 cycles, including all of the smokers' cycles and a sample of the non-smokers. To date, we selected the cycles for study and conveyed this information to the laboratory at UC Davis that originally measured the steroids. We are interested in the dynamics of FSH secretion during the transition between two cycles and around ovulation. Examining demographic variables, the non-smokers in the sub-sample are reflective of the original sample for the most part, however, they include slightly fewer Hispanics and nulligravidas. The demographic and cycle differences between smokers and non-smokers seen in the original sample are also observable in the sub-sample.

The laboratory is near completion of the FSH measurement, using an enzyme immunoassay, on 302 menstrual cycles, 110 of which are among smokers. We have been cleaning the data as it is received and have developed programs for graphical examination of the FSH levels and in relation to the steroid levels. We are developing algorithms to summarize critical aspects of the FSH measurements, such as the periovulatory peak and the slope of the curve during the luteal to follicular phase transition. The next steps will be to obtain a clean data set of FSH measurements and to link that to the other hormone and questionnaire databases. We will then conduct analyses comparing FSH parameters by smoking status. We will attempt to determine whether hormonal differences we noted previously are related to the FSH profile.

This work is relevant to many aspects of women's health, as hormone status plays a critical role through much of a woman's life. For example, short cycle has been noted as a risk factor for breast cancer and possibly early menopause. A properly functioning endocrine system plays a key role in reproduction and pregnancy of course. We are conducting biomedical research on how smoking affects this endocrine system, which may lead to interventions.

D8

More 30-day smokers accompanied with more never smokers: a myth of adolescent cigarette smoking in California, 1990-1998

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Tobacco control efforts significantly reduced cigarette consumption among adult population in California as well as in the nation. However, documented evidence on 30-day smoking behavior indicated that cigarette smoking among adolescents increased during the past decade. Smoking patterns among adolescents are more complicated than among their adult counterpart; understanding their smoking behaviors dynamics over time is important for assessing tobacco control programs. Using data of 30-day smoking alone may not be sufficient to describe the time trends of adolescent cigarette smoking at population levels.

Materials and method: With annual data collected through the California Tobacco Survey (youth part) from 1990-91, 1992 and 1993, and the California Youth Tobacco Survey from 1994-1998, this study examined the trends of adolescent cigarette smoking over time. 25,595 California boys and girls 12 through 17 years of age (50.5% males) entered the analysis. Data were collected by trained and specialized data collectors using a computer assisted telephone interview (CATI) technique. The response rate for eligible respondents ranged from 71% to 80.3% for surveys conducted in various years. Information used for statistical analyses included 30-day smoking and never smoking, two primary variables directly obtained from the survey. In additional to general statistical analyses, a graphic age-period-cohort (APC) model was used in investigating effects from the age of the subjects, the time period and the different birth cohorts on the observed time trends.

Findings: 30-day cigarette smoking prevalence increased from about 11.2% in 1990-91 to 13.17% in 1996 for boys before it declined; the same prevalence for girls increased from 9.5% in 1990 to 12.1% in 1997 before it began to decrease. Interestingly, the proportion never smokers among total respondents by year also increased during the same time period. This proportion was only 60% for boys in 1990-91; it increased to 67.5% in 1998. The same proportion for girls also increased from 67% in 1990-91 to 68% in 1998. The APC model analyses indicated that the increase in the proportion of never smokers over time was consistent with the increase in the proportion of never smokers among those who were born after year 1980, the younger cohorts in the data; while the increasing trend of 30-day smokers over time was consistent with the increased number of 30-day smokers among those who were born between 1978 and 1981, and 15 years of age and above at the survey years.

Conclusions: It is complicated to fully understand the increasing trends over time of adolescent cigarette smoking alone. It becomes even more complicated when such a trend is accompanied with an increasing trend of never smokers. Findings from this study suggest that the increasing trend of adolescent cigarette smoking in the past 9 years in California may largely be due to two cohort effects. Information from this study is useful for understanding adolescent tobacco use behavior and for tobacco control program evaluation at population level. Studies at individual and population levels should be conducted to examine the cohort effects identified from this study.

D9

Parenting and adolescent smoking behavior

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The question yet to be answered is how parents might be able to counteract the influences of tobacco advertising and of peer groups. There are considerable demographic differences in smoking behavior, which suggest that environmental influences, including parental influences, on adolescent smoking vary by race/ethnicity, educational aspirations and performance, and adolescent age. The primary aims of this project are to identify parental influences that may counteract tobacco advertising and promotion, and to identify their effects on adolescent smoking initiation in a longitudinal prospective study.

Analysis is ongoing of both adolescent (n=2,825) and parent (n=2,291) responses from a large longitudinal prospective population-based random digit dialed telephone survey. This study measured influences on adolescent smoking over a 3-year period in youth age 12-15 years at baseline to age 15-18 years at follow-up, and interviewed 1 parent, usually the mother, for each adolescent at follow-up, resulting in 2,504 adolescent-parent pairs. Preliminary findings suggest that parental reinforcement of strong expectations against smoking for their adolescent is associated with a lower rate (38.6±2.4%) of adolescent ever smoking than moderate expectations (56.4±6.6%) or weak expectations (74.4±5.4%) and is likely a key parenting practice to deter adolescent smoking throughout adolescence and into adulthood, when the risk for smoking uptake drops considerably. Other parenting correlates of adolescent ever smoking include: setting curfews, limiting nights out, close monitoring of adolescent behavior, adolescents' disposable income, and parental responsiveness. The majority of parents who smoke attributed the addictive power of nicotine as the reason they smoke (64.2±8.9%) or relapsed (78.6±10.2%) when they discussed their smoking with their adolescent. According to adolescent reports, most (62.1±10.6%) adolescents appear to accept this explanation. Most parents (78.6±2.0%) reported that parents should discuss the risks of smoking with young children, and two-thirds of adolescents (67.9±2.9%) reported that their parents had discussed the risks of smoking with them at some time. The vast majority of parents (90.7±1.7%) reported that parents should ask their adolescents about smoking that occurs among friends at least every now and then, and 47.4±3.0% reported that parents should ask regularly. While most parents (88.7±2.4%) of adolescent committed never smokers reported that their adolescent was not at risk to smoke, many (65.6±3.3%) parents of adolescents who were at risk to start or experiment further with smoking did not perceive their adolescents as being at risk to smoke. Preliminary analyses on parenting will be refined and prospective analyses of parental influences will be conducted.

Tobacco control programs can utilize results of this investigation of parental influences on adolescent smoking initiation to counteract tobacco industry influences on adolescents to smoke. This research has the ability to provide temporal evidence to argue causal effects of these influences on adolescent smoking behavior. Findings will support the development of new strategies to implement a comprehensive youth anti-tobacco program: interventions for parents and health education and tobacco marketing policy.

D10

Natural history of tobacco addiction in adolescents

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University of California, San Francisco

In a prospective cohort design, we propose to: 1) examine the natural history of nicotine addiction in adolescent smokers from experimentation to dependence including: frequency of tobacco use, changes in nicotine consumption, tobacco practices, development of perceived positive nicotine effects and development of withdrawal symptoms; 2) define nicotine addiction in adolescents by correlating rising cotinine levels over time, level of tobacco intake and presence of addictive symptoms using definitions for adults as guidelines and exploring identifiers specifically unique to adolescents; 3) examine specific social and psychological influences that contribute to the maintenance of nicotine prior to biologic addiction, specifically, positive perceived pharmacological effects of nicotine including stress reduction and weight control, negative perceived effects of nicotine withdrawal, preceding or concurrent substance use, specific situational smoking habits, peer and parental influence, chronic stress and depression; 4) explore gender and racial/ethnic influences on the natural history of nicotine addiction.

Phase I was to define the most valuable measures for the prospective study by examining the associations between adult definitions for addiction and withdrawal symptoms in small pilot groups of adolescents. Adolescents (n=63) were asked to stop smoking for three consecutive days, during which they completed daily diaries and obtained daily saliva samples. Significant predictors of adolescent tobacco dependence, as defined by correlations to craving, were retained for the final questionnaire. Cotinine levels plotted over time after smoking cessation revealed a log-linear decline.

Phase II recruitment and time 0 data collection was completed in September-October 1999. Across the seven participating schools, 74% of the parent consent forms were returned, 78% of them positive consent. 1,157 of the 2,083 eligible 9th graders completed a survey and saliva sample. Of the initial sample, 75.1% were non-smokers, 14.9% current smokers and 10.0% ex-smokers. Analysis of cross-sectional survey data revealed significant differences in depression and coping scores between smokers and non-smokers and in perceived stress between female smokers and non-smokers. Gender differences in several items were noteworthy.

Phase III longitudinal follow-up began in March 2000. Additional adolescents (n=143) were enrolled in the study. 1,229 surveys and saliva samples were completed, comprised of 72.2% non-smokers, 17.8% current smokers and 10.0% ex-smokers. Follow-up rate was 93.9%.

One of our initial analyses examined the effects of pro- and anti-tobacco advertising on non-smoking adolescents' intention to smoke. Adolescents who self-identified as never having smoked even a puff of a cigarette (n=512) completed a self-administered questionnaire which included questions on intention to smoke in the near future and tobacco advertising. Independent variables used to predict intention included exposure to, recognition of, and receptivity and attitudes towards pro-tobacco and anti-tobacco advertising. We found that receptivity to pro-tobacco advertising was associated with intention to smoke and agreement with anti-tobacco advertising was protective. Interestingly, there was no association with exposure to advertising, nor did exposure modify the effects of receptivity or agreement on intention. Future studies should focus on ways to reinforce positive attitudes towards anti-tobacco advertising and diminish receptivity to pro-tobacco advertising.

POSTER SESSIONS
Session D: Epidemiology

D11

Sex Differences in predictors of adolescent smoking cessation

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RAND

Predictors of smoking cessation from 10th- to 12th-grade were investigated in a sample of 337 male and 490 female participants in the RAND Adolescent Panel Study. This study significantly extends previous research on adolescent predictors of cessation by: a) investigating a wide range of psychosocial and behavioral variables that previous theory and research indicate may be relevant to the cessation process (pro-smoking social influences; smoking-related attitudes; other substance use and problem behaviors; bonds to school, peers, and family; and perceptions of physical and psychological well-being); b) testing for sex differences in the predictors of cessation; c) using a stringent definition of cessation in order to identify factors contributing to successful and long-term cessation; and d) testing the robustness of psychosocial and behavioral predictors of cessation when taking into account how long participants have smoked and their average daily cigarette consumption.

The sample was restricted to adolescents who reported smoking at least 11-20 times during the past year at grade 10, with 85% of them having smoked at least 3-5 times during the past month. Cessation was defined as having not smoked at all during the past year at grade 12. Forty-five (13%) males and 55 (11%) females had quit between grades 10 and 12. Results from logistic regression analyses indicated that females who quit smoking had friends who smoked less frequently, perceived less parental approval of their smoking, had weaker intentions to continue smoking, used marijuana less frequently, attended fewer different schools, were more likely to have an intact nuclear family, experienced greater peer support/connectedness, and rated themselves as physically healthier. Several of these associations could be accounted for by smoking quantity. Results for males were generally weaker and nonsignificant, although males were more likely to quit if they perceived less parental approval of their smoking. Believing that smoking has positive consequences, believing that it is not harmful, and having low resistance self-efficacy were unrelated to cessation for both sexes. Significant sex differences were found for only two predictors: delinquency and peer support/connectedness.

This study highlights a number of psychosocial and behavioral factors that are relevant to the smoking cessation process for adolescents, although it provides limited evidence that these factors substantially differ for males and females. In order to identify and target adolescent smokers who are unlikely to quit on their own, it is necessary to both pinpoint the factors that are associated with successful cessation and understand how they operate to influence behavior. This study is an initial step in this effort. Results from this study also have implications for smoking cessation programs, suggesting that programs aimed at adolescents need to take a multidimensional approach. Parental attitudes toward smoking, peer smoking, school and social bonds, and other substance use are some of the relevant psychosocial and behavioral factors that should be considered.

D12

Arterial flexibility, atherosclerosis and cigarette smoking: the Los Angeles atherosclerosis study.

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University of Southern California

Smoking is a strong risk factor for cardiovascular disease. However, the mechanisms by which prolonged exposure to tobacco smoke achieve this adverse effect is unknown. This paper explores the role of arterial wall thickening and loss of arterial flexibility as mediators of the adverse cardiovascular effects of smoking.

A cohort of 573 employed persons aged 40-60 years was randomly sampled within strata from employees of a large company. Intima-media thickness (IMT), indicative of atherosclerotic intimal thickening, was determined bilaterally over a 1 cm segment of the common carotid artery by high resolution B-mode ultrasound. Percent diameter change (%D), indicative of arterial flexibility, from minimum diastole to peak systole was also determined utilizing automated multiple frame processing of videotaped B-mode carotid images over 3 cardiac cycles. Measurements were collected over 3 examinations at 1.5 year intervals. %D is currently available only at the baseline examination.

Current cigarette smoking was adversely related to IMT in both men and women, suggesting promotion of early atherosclerosis. Increased IMT among smokers emerged in the age range 40-45 years among men, and in the age range 50-55 among women. This association is independent of other cardiovascular risk factors.

In cross-sectional analyses, arterial flexibility was strongly related to several factors in the expected direction. In a multiple regression model, %D was directly related to pulse pressure and body height, while %D was inversely related to mean arterial pressure (pressures were determined in the brachial artery by standard sphygmomanometry) and age ($p<0.001$ for all). Unexpectedly, %D was also inversely related to resting heart rate. This relation was significant in both women and men ($p<0.001$ in each), but the relation was significantly stronger in men than women (p for interaction=0.002).

Arterial flexibility was reduced among current smokers; however, the difference was small and not statistically significant.

These cross-sectional findings for arterial flexibility suggest that arterial sclerosis due to smoking may occur at a later stage of disease than atherosis. This preliminary hypothesis will be further investigated with longitudinal observation of both IMT and %D.

D13

Deaf and hard of hearing children, adolescents and young adults: what are their tobacco use patterns and unmet anti-tobacco programming needs?

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Effective strategies for preventing and reducing tobacco use among young people requires a clear understanding of the determinants and patterns of these behaviors. These patterns and determinants, in turn, vary significantly by ethnicity, culture, socio-economic and other circumstances. Our research represents a first-ever effort to gain this understanding among deaf youth and young adults, and thereby to lay the groundwork for extending tobacco control efforts to this understudied and underserved population.

We report here on a survey of tobacco use knowledge, attitudes and practices, and exposure to tobacco advertising and anti-tobacco education, among a sample of 467 deaf and hard-of-hearing (deaf/hh) children, adolescents and young adults. The respondents were recruited through thirteen schools and colleges serving deaf/hh populations in the greater Los Angeles area and in Fremont, California. Our survey used written questionnaires as well as a specially designed computer-based video technology, the Interactive Video Questionnaire (IVQJ), which administers the survey in the sign languages used most commonly by the deaf. This technology, used here for the first time among a youth population, allowed us to overcome communication, language and other barriers that have traditionally hindered inclusion of this population in data collection efforts. We also describe results of a telephone interview conducted among 45 administrators of residential and day schools for the deaf and of mainstream schools and colleges in California in which deaf/hh students are enrolled. Existing anti-tobacco programming offered in these settings for this population and unmet programming needs are identified. Finally, we outline intervention strategies for deaf/hh youth, based on our study findings, as well as input from the California Coalition of Agencies Serving the Deaf and Hard-of-Hearing (CCASDHH), that we propose to test in future research.

D14

Effect of smoking status on health and treatment of polydrug users

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Study #1: Does tobacco use in polydrug users relate to future daily functioning (SF-36) and disability? **Sample**: Community-living polydrug users (n=254) were interviewed at baseline, 1-year and 2-year follow-up. Sample included 60% men, 53% African American, 26% Latino, 18% White. Mean age was 32 years. **Measures**: Smoking status and self-reported disability at each assessment, and SF-36 measures collected at the final assessment. Urine samples permitted validation of reported drug use status. **Results**: Baseline disability rates were high but fell nearly 50% over two years. Disabilities named were similar to those reported in the general population. Change in smoking status was associated with decreased disability and improvements in general health and vitality. Respondents reporting disability reported lower daily functioning (SF-36). Stable everyday smoking was strongly associated with increased probability of positive urine tests for illicit drug use. Illicit drug use did not affect SF-36 ratings. **Conclusions**: In an observational study of polydrug users not undergoing treatment, tobacco use status was related to disability rates, to illicit drug use and to variations in daily functioning.

Study #2: Does quitting cigarette smoking help or hurt the polydrug user in treatment for illicit drug use? **Sample**: Four hundred and seven polydrug users recruited from 15 drug treatment centers in Los Angeles area. Sample included 54% women, 41% White, 32% African American and 18.5% Latino. Mean age was 36 years. Drug treatment centers included outpatient, drug-free day treatment and residential drug-free treatment. **Design**. One-year, one-wave repeated measures observational study. Only 9 percent of baseline participants were lost to follow-up. **Measures**. Smoking status, reported illicit drug use, urine test results, SF-36 scores, Hopkins Symptom Check List subscales, ASI-alcohol problems, ASI-drug problems, ASI-psychiatric problems, and questions about employment-related problems. **Results**. Almost all (95%) respondents reported some lifetime use of cigarettes. From baseline to 1-year follow-up, 15% of baseline non-smokers relapsed to smoking and 14% of baseline smokers quit their habit. At follow-up, former smokers reported fewer psychiatric problems than current smokers. Changing smoking status was not associated with changes in illicit drug use, as measured by urine drug test, but was associated with reductions in self-reported heroin. On the other hand, changing smoking status was associated with increased psychiatric problems and more employment-related problems. Stable former smokers consistently reported healthier outcomes than either stable smokers or status changers as reflected in somatization, obsessive/compulsive behavior, depression and anxiety scores. **Conclusions**. In polydrug users undergoing treatment for illicit drug use, long-term abstinence from tobacco use by former smokers was associated with consistently more favorable health outcomes than either stable everyday smoking or changing one's cigarette smoking status during treatment. Treatment for illicit drug use may be more effective in polydrug users who have quit smoking cigarettes prior to entry to treatment for drug abuse. **Future direction**: These and other results will inform future proposals for more effective tobacco abstinence programming targeted to the 75% of drug users who typically smoke cigarettes. If effective, such programming should save lives and reduce future medical care costs.

Session E: Cancer: Mechanisms/Diagnosis/Treatment

E1

Folate status is affected by smoking and only some folate indices improve following vitamin supplementation in smoking compared to non-smoking men.

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Although evidence demonstrates that cigarette smoking depletes the body's antioxidant nutrients (in particular vitamin C), little is known about the impact of smoking on other water-soluble vitamins, such as folic acid. Folic acid is important because it plays critical roles in DNA synthesis and repair (processes that are essential to normal cell division) and in modifying DNA to turn genes on or off at critical times. We evaluated whether or not smoking and a modest vitamin supplementation regimen alters folate indices in men with low fruit and vegetable intakes. Since smokers typically consume poorer diets compared to nonsmokers, we carefully screened our subjects to minimize dietary differences at the study start. Seventeen nonsmokers and 18 smokers who habitually consumed only ~2 servings/d of fruits and vegetables took a daily, combined supplement containing 272 mg vitamin C, 31 mg a-tocopherol acetate and 400 mg folic acid, while 22 nonsmokers and 20 smokers took a placebo for 90 days. The supplement contained only modest amounts of vitamins and was designed to mimic what the subjects would have consumed if they had eaten a better diet, i.e., one containing the National Cancer Institute's recommended 5-9 daily servings of fruits and vegetables. Blood samples were collected at baseline and at the end of the study for determination of blood plasma folate and homocysteine (a functional measure of folate nutriture).

Baseline data revealed that the smoking men had significantly higher plasma folate compared to nonsmokers (13.7 $\pm$ 7.9 vs. 10.0 $\pm$ 5.4 nM; $p<0.05$). Homocysteine concentrations were not significantly different between the 2 groups. Blood plasma folate increased from 11.1 (mean) $\pm$ 5.6 (SD) to 27.4 $\pm$ 10.3 nM in supplemented nonsmokers ($p<0.0001$) and from 15.8 $\pm$8.5 to 30.0 $\pm$ 15.0 nM in supplemented smokers ($p=0.0002$), whereas blood plasma folate in both placebo groups did not change. There was a 23% increase in food folate intake during the study in the nonsmokers' placebo group, but this was not accompanied by an increase in plasma folate. Plasma homocysteine concentrations declined by 20% only in the nonsmokers' supplemented group ($p=0.03$).

These findings delineate the negative impact of smoking on certain aspects of folate nutriture, in a population of men with similar dietary intakes. It also demonstrates the benefits of vitamin supplementation for improving plasma folate concentrations in both smoking and nonsmoking men. The lack of a significant decline in homocysteine concentrations in smokers suggests that the effects of smoking on a more functional measure of folate nutriture cannot be overcome by dietary supplementation alone. Small, individual differences in the gene encoding for the folate dependant enzyme, methylene tetrahydrofolate reductase exist, however, and could explain the lack of a significant decline in homocysteine in the smoking group. We plan to genotype all subjects for the common mutation in this gene in the near future. These findings lead us closer to understanding, and eventually minimizing, the mechanisms by which cigarette smoking impacts nutrition and leads to cancer development.

E2

Polymorphisms in the AHR, ARNT and CYP1A1 genes and their potential role in cigarette-induced lung cancer.

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Cigarette smoking can induce CYP1A1 in the lung. Induction requires the aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT) proteins. Lung samples from seven of 75 Finnish patients who smoked until the time of surgery exhibited absent or low levels of CYP1A1 protein, mRNA and enzymatic activity, suggesting that these individuals might be genetically non or poorly inducible for CYP1A1. All seven lung samples expressed normal levels of AHR mRNA and ARNT mRNA, indicating that they did not carry inactivating polymorphisms in the 5' upstream regulatory regions of these genes. Sequencing of cDNAs encompassing the complete coding regions of *AHR* and *ARNT* identified a previously known codon 554 polymorphism in *AHR*, which was present in the homozygous state in one individual. This polymorphism, which leads to an amino acid substitution, has been reported either to have no effect or to enhance CYP1A1 induction. Previously unreported silent single nucleotide polymorphisms were identified in codon 44 of *AHR* and codon 189 of ARNT. 1500bp of genomic sequence from the 5' upstream regulatory sequence of the *CYP1A1* gene was also sequenced in the non-inducible individuals. A nucleotide substitution polymorphism at position -459 was detected in the heterozygous state in two individuals. This polymorphic site does not reside in any known regulatory sequence. The complete *CYP1A1* coding sequence and intron/exon boundaries were then sequenced. None of the non or poorly inducible individuals exhibited any polymorphisms, either homozygous or heterozygous, compared with representative inducible individuals. Thus no polymorphisms in the *AHR, ARNT* or *CYP1A1* genes were identified that could be responsible for the non/low inducibility phenotype observed.

CYP1A1 in the placenta is usually highly inducible by maternal cigarette smoking. Placentas were obtained from two mothers who smoked up until the time of delivery and yet whose placentas exhibited very low or absent CYP1A1 expression. We hypothesized that corresponding fetuses would carry polymorphisms in the *AHR, ARNT* or *CYP1A1* genes leading to non- or poor-inducibility. Four placentas from smoking mothers were also studied that express exceptionally high levels of CYP1A1, suggesting that they may possess polymorphisms in the one or more of the above genes leading to enhanced functionality. The *AHR* and *ARNT* coding regions from these individuals were sequenced. The same polymorphisms in *AHR, ARNT* and *CYP1A1* were identified that we previously identified in the lung samples. It is unlikely that these polymorphisms explain the above phenotypes.

We are currently looking for polymorphisms in a large cohort of lymphocyte samples, some of which are non-inducible for CYP1A1 in vitro.

POSTER SESSIONS
Session E: Cancer: Mechanisms/Diagnosis/Treatment

E3

Tobacco smoke induces SINE RNA stress response

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Cells cope with various stresses, such as tobacco smoke exposure, by mounting a specialized response to aid in recovery. The classic stress proteins, called heat shock proteins (HSPs), were originally discovered upon subjecting cells to high temperatures. Later it was determined that HSPs are in fact induced by a wide variety of stresses including exposure to tobacco smoke, heavy metals, and other conditions that cause cellular damage.

RNA polymerase III directed SINE transcription is also induced by wide range of stresses. We have characterized this novel stress response and have shown that it is conserved in evolution in organisms as diverse as mammals and insects. Heat shock induces both SINE RNAs and HSP70 mRNAs. Furthermore, many other cellular stresses including heavy metals, translational inhibitors, alcohols, virus infection, etc. induce both the SINE RNAs and the HSPs. SINE induction occurs as a normal part of the cell's stress response to these harmful environmental conditions. We further discovered that over expression of Alu RNA, the human SINE, stimulates the synthesis of a reporter protein suggesting that these RNAs may have a role in regulating translation during stress recovery.

In this current study, we find that tobacco smoke induces Bm1 RNAs in silkworm larvae one day after exposure and persists thereafter until the larvae die from exposure. Due to the high nicotine content of the aqueous cigarette tar (ACT), we also separately tested aged solutions of catechol and hydroquinone, two of the major constituents of ACT. Both hydroquinone and catechol induce a transient increase in Bm1 RNA in silkworm larvae. However, none of the tobacco related chemicals induce HSP70 mRNA. This study demonstrates how different stress treatments induce distinct components of the stress response each having the potential to be developed as a biomarker for the early detection of cellular damage.

Presently, the effect of ACT and other components of tobacco smoke on the Alu RNA and the HSP stress responses is being investigated. The goals of this investigation are to learn the role of Alu RNA in stress recovery and to distinguish between different stress induced pathways activated by tobacco smoke and its components.

E4

Disruption of protein phosphatase 2A subunit interaction in lung cancer with a mutation in the Aa subunit gene

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Smoking causes lung cancer through mutation of genes that are involved in controlling the growth of lung cells. Two classes of genes are important in growth control: Genes that stimulate growth (oncogenes), and genes that inhibit growth (tumor suppressor genes). The former become activated by mutation whereas the latter become inactivated. Mutation of both types of genes contributes to the development of cancer.

For many years, our laboratory has investigated protein phosphatase 2A (PP2A), which controls the function of other proteins by removing phosphate residues from the amino acids serine and threonine. PP2A exists in cells as core enzyme composed of a catalytic C subunit and a regulatory A subunit, and multiple holoenzymes consisting of core enzyme to which one of several B subunits is bound. The A and C subunits both exist as two isoforms (α and β), whereas the B subunits fall into three families designated B, B', and B'', which are unrelated by protein sequence. It was recently discovered that the regulatory subunits Aα and Aβ are mutated in lung cancer, indicating a role of Aα and Aβ in tumor suppression. Based on our recent mutagenesis experiments with the Aα subunit, we hypothesize that the lung cancer-associated mutations in Aα and Aβ abolish binding of specific B subunits to the corresponding core enzymes, resulting in loss of growth control.

The goal of our research is to identify the B subunits that normally bind to Aα and Aβ-containing core enzyme, and to investigate how the mutations in the Aα and Aβ subunits, which were found in lung cancer, affect binding of regulatory B, B', or B'' subunits to core enzymes. Binding experiments were carried out according to a method developed in our laboratory. Here we report results with the Aα mutant Glu64->Asp in the N-terminal region of the protein. We found that this mutant was defective in binding the B' subunit but normal in binding the B, B'', and C subunits. This finding indicates that lung cancer cells with the Glu64->Asp mutation cannot form AB'C holoenzyme, suggesting that AB'C may play a role in tumor suppression. In the future, we plan to study the binding properties of other lung cancer-associated mutants in the Aα and Aβ subunits.

Our work is relevant to lung cancer, since it is highly likely that smoking causes mutations in the Aα and Aβ subunits resulting in loss of the presumed tumor-suppressing function of PP2A. It is conceivable that in the future, based on our studies, drugs can be found that revert the effect of Aα and Aβ mutations in lung cancer, i.e. drugs that bind to core enzyme and exert the same effect on enzyme activity as the tumor-suppressing B subunit. Since we are dealing with an enzyme, searching for drugs in a natural product or synthetic compound library is a worthwhile and realistic goal.

Session E: Cancer: Mechanisms/Diagnosis/Treatment

E5

Tissue distribution and macrolecular binding of [^{14}C]-Nicotine and [^{14}C]-Hydroquinone from passive smoke in AJ/1 mice.

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Lawrence Livermore National Laboratory

Nicotine and hydroquinone (HQ) are two of many chemicals released from the burning end of cigarettes. These compounds have not been quantitatively assessed for health effects at passive smoke concentrations. HQ is produced from the coal tar and nicotine can be converted to nitrosamines in tobacco smoke which has been shown to cause lung and other cancers. The aim of this project is to study the tissue distribution, protein binding, and DNA binding of [^{14}C]-nicotine and [^{14}C]-HQ at levels equivalent to what is found in the passive smoke from a single cigarette.

[^{14}C]-nicotine was administered to AJ/1 mice at a dose of 125 µg/kg body weight. Tissues, protein and DNA were analyzed for [^{14}C]-nicotine content between 0 - 48 hr post exposure. Tissues were analyzed by liquid scintillation counting; DNA and protein were analyzed by accelerator mass spectrometry. The highest levels of [^{14}C]-nicotine equivalents were found in the plasma, testis and liver, followed by lung and brain. [^{14}C]- nicotine concentration peaked between 15 - 60 minutes and declined thereafter in all tissues analyzed. Similarly protein and DNA adduct levels were highest at 1 hr in the liver tissue. However, in the testis only protein adducts were detectable and peaked between 15 – 60 min post exposure. No DNA adducts were detectable by accelerator mass spectrometry. Dose–response assessments at 1 hour showed that liver DNA adducts and tissue available doses increased with increasing nicotine dose up to doses of 500 µg/kg body weight, but the dose response is not linear. Protein adducts were greater than DNA adducts in all tissues analyzed. These data show that nicotine is bio-available at passive smoking doses and cause damage to macromolecules such as protein and DNA. Similar studies are presently underway with hydroquinone. Overall this study will help to understand the interactions of both nicotine and tar in the initiation of carcinogenesis from passive smoke. This work performed under the auspices of the US DOE by LLNL (W-7405-ENG-48) and supported by the UC Tobacco-Related Diseases Research.

E6

Novel imaging methods for diagnosing tobacco related cancers

Macovski, Albert
Stanford University

During the three and a half years of this grant, we have made excellent progress towards these goals. We successfully recorded our first human in vivo human images this fall. We are still debugging minor image artifacts, but the image quality is quite good with 1 mm resolution and image aquisition time of 56 seconds. These were human wrist images with 0.4 T polarizing magnet field and 1 MHz center frequency. This is a clear and successful proof of concept of Prepolarized MRI. In the final 3 months of the grant period, we plan to improve the shim of our 31-cm-bore readout magnet, finish construction of the 45-cm-bore polarizing magnet, and develop a larger polarizing switching supply.

Studies have shown that non-drinking smokers have a 4- to 13-fold increase in risk for this cancer. MRI is currently the key clinical tool to indicate surgery over chemotherapy. Our PMRI system could reduce the cost of treatment staging. Ultimately, we hope that a PMRI scanner could be inexpensive enough that society could justify screening high-risk patients. Screening is now routine for diagnosing colon cancer, and early diagnosis is the best predictor of survival. Hence, PMRI could improve the survival rate and reduce the total societal costs of treating tobacco-related tumors of the head and neck.

POSTER SESSIONS
Session E: Cancer: Mechanisms/Diagnosis/Treatment

E7
Detection of occult metastases in patients with lung cancer

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Surgery, with or without chemotherapy, remains the only potentially curative therapy in patients with primary lung cancer. Because treatment failures are common in patients thought to be cured, the primary goal of this study is to determine whether or not the presence of occult (hidden) metastases in the bone marrow and lymph nodes in these patients will identify those whose disease is most likely to recur. In addition we are studying pleural lavage fluids collected both immediately before and after primary tumor resection and analyzing them from the presence of tumor cells. To this end we are in the process of collecting bone marrow and lymph node specimens from multiple institutions around the United States and examining them for the presence of early disseminated tumor cells using two extremely sensitive detection methods.

To date we have received over 120 bone marrow and pleural lavage fluid specimens from over 16 different institutions and lymph node specimens from 49 patients who were lymph node negative by routine methods of assessment from 9 different institutions. Preliminary analysis by immunohistochemistry (IHC) of the first bone marrow specimens that we obtained shows that occult bone marrow metastases were found in 4 of the 23 cases tested (17%). Of 14 cases that were diagnosed as being lymph node negative by routine histologic analysis, 3 (31%) were found to have occult metastases by IHC. The number of institutions enrolling patients is increasing at a rapid rate.

As the study progresses we will continue to accrue bone marrow, pleural lavage and lymph node specimens and analyze them for the presence of occult metastases using IHC. In addition, we will be testing the samples using alternate sensitive methods (molecular method; RT-PCR). We will then compare the results of each of the methods with each other, with the status of the disease at the time of operation. We will also assess the relationship between occult bone marrow and occult lymph node metastases to determine whether these two compartments represent separate, independent prognostic factors and which, if either, is the better predictor of disease outcome. Ultimately, we will compare the results with the incidences of disease recurrences and disease related deaths. In this way we will be able to determine which is the better method to predict the future behavior of the disease and therefore which patients would most benefit for additional anticancer therapy. Finally, we will study the individual cancer cells in the positive specimens to analyze their dormancy status. This dormancy status may have a significant impact on the treatments used for patients who suffer a recurrence of their disease.

E8
The angiogenic role of Interleukin-8 in lung cancer

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Smoking is a major risk factor for lung cancer, and in spite of medical advancements the prognosis of lung cancer patients remains poor, primarily because of metastatic dissemination of the tumor prior to its diagnosis. Because of the poor prognosis of metastatic lung cancer, novel strategies are necessary for the development of therapies that provide better survival and quality of life for those afflicted. A promising new strategy is the development of anti-angiogenic factors, among them the IL-8 family of chemokines. Although it has been known for several years that IL-8 and gro-a are angiogenic factors, they have not found proper attention in this respect, in spite of the fact that lung cancer cells produce IL-8.

IL-8 is better known for its role in acute inflammation, where it attracts neutrophils into an area of tissue injury. Human leukocytes express two kinds of IL-8 receptors, the CXCR1, which mediates leukocyte migration and the CXCR2, whose function is poorly understood. Because of experimental difficulties in detecting IL-8 receptors on endothelial cells *in vitro*, it had been assumed that these receptors are lost in culture. *In vitro* experiments using human cells are essential, however, because *in vivo* experiments in mice have limited meaning, since mice express only one IL-8 receptor, which more closely resembles the CXCR2. Thus murine models reflect poorly the conditions present in humans. Here we show that cultured human endothelial cells express both the CXCR1 and CXCR2, but that the CXCR2 mediates the prolonged IL-8 mediated responses in endothelial cells. Blocking antibodies to the CXCR2 inhibited IL-8 or gro-α induced cell retraction and gap formation between endothelial cells as well as haptotaxis on collagen in the presence of a gradient of IL-8 or gro-α, while antibodies against the CXCR1 failed to show a blocking effect. Furthermore several interventions in the downstream signaling cascade of the CXCR2 (dominant negative rac, inhibition of PI-3 kinase) inhibited the IL-8 mediated response. These results suggest that inhibition of the CXCR2 on endothelial cells may block the angiogenic effect of IL-8 without interfering with the IL-8-mediated immune protective role of the CXCR1 on neutrophils. Blocking antibodies against the CXCR2 or small molecular weight compounds that specifically inhibit the CXCR2 are viable options as anti-angiogenic strategies in lung cancer in the future.

Session E: Cancer: Mechanisms/Diagnosis/Treatment

E9

Study of novel metastasis inhibitors in lung cancer

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Metastasis is responsible for a high proportion of lung cancer morbidity and mortality. During that process, certain interactions between circulating tumor cells and host elements facilitate eventual deposition and uptake of metastatic cells in distant tissue sites. These include tumor cell – platelet interactions as well as tumor cell – endothelial interactions. The expression of certain tumor cell-surface glycosylated molecules which bear characteristic terminal sugar configurations facilitates attachment between such "ligands" on tumor cells and the selectin class of adhesion receptors on the surface of platelets and endothelia. A growing literature shows that such selectin-mediated interactions are important in mediating early steps in metastasis. Our laboratory has recently demonstrated that novel disaccharide compounds can potently block expression of such terminal sugars on malignant cells, and thereby reduce their adhesive potential. The goal of this research is to investigate the effect of these glycosylation inhibitors on the adhesive and metastatic abilities of human lung cancer cells.

We have made a number of observations which demonstrate the efficacy of our compounds in blocking the adhesive and metastatic potentials of solid tumor cells. Micromolar levels of these compounds appear to inhibit adhesion of tumor cells to immobilized as well as free selectins in a dose-dependent manner. Initial studies on tumor cells from human lung, colon, lymphoma, and prostate cancers showed approximately 10 – 20 fold increased potency over that of an historical monosaccharide inhibitor. Using metabolic labelling studies, we have demonstrated that our novel inhibitors appear to reduce those specific tumor cell-surface sugars responsible for adhesion by diverting the cell's glycosynthetic enzymes away from endogenous glycosylated intermediates and onto the disaccharide "acceptor" compounds. This method of reducing the tumor cell's selectin binding capacity also appears to potently inhibit adhesion of tumor cells to human platelets and microvascular endothelia *in vitro*. We have treated colon tumor cells with micromolar quantities of our inhibitors, and altered their metastatic ability in both short- and long-term biodistribution studies in mice. Currently, we are extending such *in vivo* models to include lung tumor cell lines as well. We are also in the process of investigating the pharmacology of our inhibitors in mice in order to ultimately address both the efficacy and safety of systemic delivery of these compounds. Finally, the growth of metastases depends on their ability to attract a microvascular supply (angiogenesis), and we hypothesize that tumor angiogenesis may also be affected by inhibiting the selectin ligands on lung tumor cells. Studies using intravital microscopic technology are planned to examine the effect of our inhibitors on tumor angiogenesis *in vivo*.

This research is especially relevant to lung cancer since a high proportion of lung cancers express the characteristic terminal sugars we target, and expression of these characteristic structures on tumor cells correlates with mortality. We hope to ultimately extend these or related compounds as therapeutic agents against this high-mortality tobacco-related disease.

E10

Studies of novel antitumor agents for lung carcinomas

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Our laboratory, under sponsorship provided by the Tobacco-related Disease Research Program, identified a novel class of agents called Acylfulvenes that displayed toxicity towards a variety of cancers including non-small cell lung tumors. We identified one analog that displayed marked anticancer activity both *in vitro* and in a drug-resistant lung carcinoma xenograft model. This drug, Irofulven (HMAF, MGI-114, or NSC 683863), has completed phase I and phase II human trials against several solid tumor types. Based on promising results from these early trials, the Phase III (final) human trials are scheduled to began this winter under sponsorship of a pharmaceutical company and the National Cancer Institute.

Despite these promising clinical results, little is known about the action or metabolism of Irofulven. To aid in future development of this class of compounds we needed to identify proteins involved in cellular accumulation and metabolism. Completion of these goals would allow development of an in vitro screening system to rapidly identify promising analogs, and allow for sophisticated methods such as computer modeling.

As part of this process we used phage display to identify peptide sequences capable of binding to Irofulven. Subsequent analysis of these peptide fragments suggested that Irofulven may interact with DNA binding domains such as that found on the enzyme Topoisomerase I. This suggested that Irofulven might have a synergistic therapeutic effect when combined with Topoisomerase I inhibiting agents. This was confirmed using cell culture and lung carcinoma xenograft models. Indeed, in an initial phase I trial, a patient with Non-Small Cell Lung (NSCL) cancer demonstrated response to the combination therapy of Irofulven and Irinotecan.

POSTER SESSIONS
Session E: Cancer: Mechanisms/Diagnosis/Treatment

E11

Developing New Vaccine Approaches for Lung Cancer

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Lung cancer is a major cause of smoking-related death and new approaches to treatment are needed. The primary goal of our study is to test two immune-stimulating drugs, GM-CSF and IL-4, for their ability to enhance the immune response to lung cancer. Dendritic cells (DC) are responsible for stimulating immune responses, including the response against cancer. However, lung cancers escape the immune system by turning off the maturation and function of DC. We have developed a mouse model in which infusion of GM-CSF and IL-4 generates DC in the spleens and immune organs of treated mice. This treatment, and the resulting increase in DC, is being evaluated for its ability to inhibit lung-tumor growth. Finally, by combining this treatment with a gene therapy vaccine, we hope to develop a treatment capable of completely eradicating established cancers. To address this issue we have developed a genetically engineered common cold virus (an adenovirus) which can be co-administered with these drugs as a cancer vaccine.

Our results demonstrate that GM-CSF and IL-4 generate functional DC *in vivo*. Specially designed osmotic pumps were used to continuously deliver GM-CSF and IL-4 to mice for 7 days (10 mg/day of each cytokine). This treatment significantly increased spleen weight and total spleen cell number by up to 4-fold. Spleen cells expressing the DC marker CD11c increased from 5 % in control animals to 15 % in GM-CSF treated mice and 20% in mice treated with both GM-CSF and IL-4. Both the myeliod ($CD11c^+/CD11b^+$) and lymphoid ($CD11c^+/CD8a^+$) DC subpopulations were increased, with the greatest effect on both populations observed with the combination of GM-CSF and IL-4 (8 fold increase in myeloid DC and 2 fold increase in lymphoid DC). Treatment was associated with architectual disruption of the normal spleen structure due to the rapid increase in spleen size and cell number. A significant increase in MHC-I and MHC-II expression was detected on DC from treated mice. These DC also had greater activity in endocytosis and pinocytosis assays, as well as higher T cell stimulatory activity in the MLR assay. Taken together these results show that functional DC are generated *in vivo* when animals are treated with GM-CSF alone or in combination with IL-4.

Preliminary studies demonstrate that administration of GM-CSF and IL-4 slows the growth rate of implanted tumors. In ongoing experiments we are combining this drug therapy with a specific tumor vaccine. We anticipate that the increased numbers of DC will allow animals to respond optimally to the vaccine and result in complete tumor eradication. A recombinant adenovirus expressing the b-gal transgene is being used to test this hypothesis.

In summary, our study confirms that GM-CSF and IL-4 can be used to increase the number of functional dendritic cells and enhance anti-tumor immune responses. We are now testing different strategies to take advantage of this effect and develop an effective immunotherapy for lung cancer.

E12

Tobacco-related cancer prevention by Vitamin A derivatives

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Molecular Medicine Research Institute (M.I.D., C.W.L., G. C.); SRI (P.D.H.); The Burnham Institute (J. G., S.K.K., X. Z.); Wayne State University (Y. Z., J.A.F.); John D. Dingell VA Medical Center (J.A.F.)

Lung cancer is the leading cause of cancer death of Californians. Unfortunately, most lung cancers are usually not detected until after metastasis has occurred. Despite surgery, irradiation, and chemotherapy, early-stage disease has a five-year survival rate of only 42% and late-stage disease a rate of 14%. Tobacco use and exposure are the main cause of lung cancer, and use among young people and women is increasing. Therefore, the need for more effective methods to prevent and treat lung cancer is urgent.

Recent studies indicate that induction of apoptosis (a natural form of cell death) in cancer cells may enhance cancer prevention in patients at risk for developing this disease. We found that a derivative of vitamin A, namely, a retinoid called 6-[3′-(1″-adamantyl)-4′-hydroxyphenyl]-2-naphthalenecarboxylic acid (abbreviated AHPN) inhibits lung cancer cell growth and induces apoptosis in these tumor cells. Our studies indicate that AHPN exerts its anticancer activity by a mechanism uncharacteristic of retinoids. This mechanism produces an advantage because the toxic effects of AHPN caused by its retinoid activity may be able to be removed in new derivatives.

We have identified such a compound, which we named MM11453. This compound is able to inhibit lung cancer cell growth in culture in both retinoid-sensitive and insensitive cell lines. Interestingly, we recently discovered how this compound initiates the process of apoptosis. Mitochondria are miniscule organs in cells that contain the energy-producing machinery necessary for cell survival. When this machinery is stopped or a cell is so stressed by external factors that it can no longer function, mitochondria assume the role of cell executioners by initiating apoptosis. They do so by releasing cytochrome c, which activates enzymes that degrade cell proteins. Both AHPN and MM11453 induce cytochrome c release by causing a protein called TR3 that is usually present in the nucleus of healthy cells to move to the surface of the mitochondria. There, TR3 interacts with certain gatekeepers so that cytochrome c is freed. MM11453 is does not function as a normal retinoid that initiates retinoid-responsive genes to function.

Session F: Community and Clinical Interventions

F1

Maximizing school, community, and parental participation in school-based tobacco research programs

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The development of successful school-based smoking programs for youth necessitates access to the classroom where curricula may be initially piloted, refined, then finally delivered and evaluated for effectiveness. Increasingly, however, school recruitment has become a daunting task due to the many curricular and extracurricular demands placed on school districts, principals, and teachers. Even the successful recruitment of schools is but one step in the process of conducting smoking prevention or cessation research. Individual teachers must find space in their busy lesson plans for multi-lesson curricula as well as provide support for these programs. When active consent is sought, parents must understand the nature and process of the research and feel comfortable enough with the contents of the consent form to sign and return it to the school or investigator. In the case of longitudinal research, the demands on schools, parents, and students can increase significantly. Despite the complex issues facing those who conduct school-based research, there is a paucity of literature offering systematic, pragmatic solutions to the problem of recruitment and subsequent successful execution of research studies.

Over this past pilot year, we have grappled with the difficulties inherent in this process as we recruited schools for our Pacific Rim smoking prevention trial with middle-school students. An additional challenge has been that of gaining access to ethnically and culturally diverse parents, many who are non-English speaking. From our work with educators, parent groups, community-cultural consultants, psychologists, and social workers, we have created a multi-faceted approach to address the unique issue of maximizing participation in school-based tobacco research. Utilizing a complex flowchart to track all aspects of the research process, our three-pronged methodology identifies possible problem areas at the school, community, and parental level along with appropriate interventions. Ultimately, our goal is to foster collaborative relationships with schools, communities, and parents and while developing effective smoking prevention programs for widespread dissemination.

Given the importance of school-based research to address smoking prevention, cessation, and other health concerns impacting California's youth, our approach to increase participation in school-based research comes at an important time.

F2

Development of a tobacco prevention curriculum for Pacific Rim 6 th graders

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Research has shown that tobacco prevention programs can delay onset of tobacco use through normative restructuring and counteracting social influences. However, most of the research to date has been conducted on middle class, Anglo students. More research is needed to determine protective and risk factors for smoking among different ethnic groups and to determine the efficacy of program components and modes of delivery among those ethnic groups. This study seeks to develop a model for culturally tailored curriculum development and to produce a multicultural curriculum targeting California's two emerging ethnic majorities, Asian and Latino.

Over the last year, a series of quantitative and qualitative studies have been conducted. Survey results, focus groups, individual interviews, ethnographic field studies, activity tests and a learning styles evaluation have provided the basis for curricular tailoring. For example, early survey data suggest that cultural values, including Filial Piety (a child's duty to respect parents) and Simpatia (importance of smooth, harmonious social relations) may be protective against smoking indicating the importance of placing particular emphasis on social consequences of tobacco use within the curriculum. Also, focus group and individual interview data have indicated that 6th graders do not yet have a strong expressed sense of ethnic identity and do not perceive strong differences based on ethnicity among their peers. This data led us to reject the notion of developing an ethnically specific curriculum that may be more appropriate at later age groups.

Further, a learning styles preferences evaluation found preferences varying by ethnic group. Activities varying by learning style were administered in 32 classrooms at 8 schools to approximately 900 students. Asian students had a higher perceived knowledge gain in an individual context versus a group context. Latinos reported a higher likeability of the activities overall more than Asians, regardless of learning style. And Filipinos reported a preference for a kinesthetic learning style over visual learning style. These results support the hypothesis that tailoring tobacco prevention activities by learning style for different ethnicities would be beneficial and that a multicultural curriculum should appeal to a variety of learning styles.

Lastly, cultural experts from academics, education and the community have assisted in the interpretation and integration of study results into the curriculum. This research is necessary for the advancement of tobacco prevention curricula that addresses the changing demographics of California's youth, as well as to aid in the future development of culturally tailored curricula.

POSTER SESSIONS
Session F: Community and Clinical Interventions

F3
Predictors of CHA Success in Tobacco Control Interventions

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San Diego State University

Community Health Advisors (CHAs) are indigenous lay health advisors who are members of existing social networks in the community. With training, these individuals can create health awareness, disseminate health information and support behavior change in their communities. Little data is available examining characteristics of successful CHAs, or *promotores*. The Tobacco Control in Latino Communities (TCLC) Center trained 35 *promotores* to conduct interventions to 1) develop and evaluate a *promotor*-based smoking cessation program for Spanish-speaking adult smokers, and 2) develop and evaluate a *promotor*-based behavioral problem-solving approach to reducing environmental tobacco smoke (ETS) exposure among low-income Latino children. *Promotores* were given self-report instruments before and after training that included measures of various psychosocial constructs relating to behavior change as well as general self-esteem, general self-efficacy, and demographic questions. Of the 34 women and 2 men recruited for this study, 65% had some high school education, 65% report household income <$1,500/month, and 88% were originally from Mexico. The mean age of participants was 44 years of age. This poster will describe indicators of *promotor* "success" for each intervention described, and predictors of success will be reported. Identification of such predictors can aid in future recruitment of CHAs for use in health promotion programs.

F4
Proyecto sol: smoking cessation for latinos using community health advisors

Talavera, Gregory A.; Elder, John P.; Woodruff, Susan I.
San Diego State University

Although studies have demonstrated the need to develop culturally appropriate cessation programs that take into account the specific cultural beliefs and experiences of the Latino smoker, few strategies have been developed to help Latinos quit smoking. The present study recruits Spanish-speaking Latino smokers living in San Diego County and randomizes them into either an intervention group or a comparison group. The intervention group receives a culturally sensitive, Spanish-language 4-month program delivered in the home by community health advisors, or *promotores*. Participants in the comparison group are referred to the Spanish-language California Smokers' Helpline via mailed postcards.

Demographic data on 312 enrolled participants indicate that the majority were born in Mexico, about half are women, the average age is 43 years, and most are of a moderately low acculturation level. In terms of smoking characteristics at entry into the study, the average age of initiation was 16 years, 90% consider themselves daily smokers consuming an average of 11-15 cigarettes per day, and 81% have tried to quit in the past.

Preliminary data on 209 participants with pre- and post-intervention survey data show that the intervention group had significantly higher self-reported past-week abstinence rates at post-test (26%) than the comparison group (13%). However, mean carbon monoxide readings improved slightly in *both* groups. Further, both groups reported statistically significant improvements in the amount smoked per day and the number of recent quit attempts. Smoking/cessation-related knowledge gain was significantly higher in the intervention group, although several cessation-related attitude measures (e.g., intentions, outcome expectancies, self-efficacy) showed no significant differential group change. Post-test data collection is continuing, as is a 12-month follow-up assessment to evaluate longer-term changes associated with this novel promotor-based smoking cessation program.

F5

Ambiente fresco: reducing environmental tobacco smoke in latino children

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Environmental Tobacco Smoke (ETS) has been associated with a variety of illnesses in children, including increased rates of respiratory illness, middle ear infections, decreased lung function, asthma, and Sudden Infant Death Syndrome. Many children, however, continue to be exposed to ETS in the home, often by smoking parents or relatives who may not fully realize the possible harm to their children. Having a low income also is a well-replicated risk factor for smoking. Because a substantial number of Latinos living in San Diego County reside in low-income households, Latino children may be at especially high risk for ETS exposure.

This randomized controlled trial is evaluating a culturally sensitive, behavioral problem-solving approach to reduce ETS exposure among young Latino children in San Diego County. Intervention efforts are directed toward a key member (e.g., the mother) of the household in which the child lives. Bicultural, bilingual lay community health workers, known as promotoras, are conducting the intervention using contracting, shaping, positive reinforcement, and stimulus cues to assist the household contact in achieving ETS reduction goals. Participants (n=143) have been randomly assigned either to the control group or to the 4-month intervention group. Parent's reports of the child's exposure to ETS in the home, and the child's hair nicotine/cotinine levels are being collected at baseline and at three post-intervention time points during a one-year follow-up.

Preliminary analyses for 99 families with baseline and immediate post-intervention data indicate that, on average, families exposed their children to significantly less environmental tobacco smoke over time. However, approximately the same reduction was seen across both groups with intervention and control families reducing their children's past-month ETS exposure by 19-20 cigarettes. The intervention did, however, affect several knowledge/attitudinal measures as indicated by significant group-by-time interactions. Knowledge related to ETS exposure, self efficacy regarding one's ability to reduce the child's ETS exposure, and positive expectations related to the benefits of reducing ETS exposure increased in the expected direction in the intervention group but remained essentially unchanged in the control group. Follow-up data collection is continuing to evaluate long-term changes associated with this novel promotora-based ETS reduction program.

F6

Effectiveness of two different regimens in a smoking clinic

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Several studies have examined the efficacy of different smoking cessation therapies. However, few studies have assessed their effectiveness in everyday clinical practice. We analyzed the effectiveness of bupropion versus bupropion plus nicotine patch in a routine clinical setting at the Sepulveda VA's Smoking Cessation Clinic (SCC). Our primary goals are to determine the effectiveness of each regimen at the end of treatment and at 6 months and to evaluate the differences in cost between the two regimens.

All patients referred to the SCC were asked to participate in the study. Consenting patients filled out a detailed baseline survey covering smoking history, health habits, health status, anxiety and depression. The SCC consisted of 7 visits over 2 months, with patients receiving individual counseling from both a health educator and clinical pharmacist at each visit. Analyses were conducted based on the initial treatment assignment.

The SCC has received 333 patient referrals since the start of the enrollment period. Of these, 203 patients (61%) attended at least one session and 164 (79%) consented to participate in one or more parts of the study. Of the 164 study patients, 73 are in the bupropion treatment group, 68 are in the combination treatment group, and 23 receive nicotine patch alone (due to medical contraindications to the bupropion).

Baseline survey data indicate that 95% of study participants are male, 74% white. On average, they were smoking 26 cigarettes a day before starting the SCC. The mean age at which participants first started smoking was 15. Over 91% had attempted to quit at least one time prior to their current quit attempt, with over 71% stating that their main reason for deciding to quit was health reasons. Approximately 42% of participants had used the nicotine patch in the past; about 30% had used bupropion for smoking cessation in the past.

The 2-month SCC was successfully completed by 33% (18/55) of the combined therapy group, 24% (14/59) of the buproprion group, and 18% (4/22) of the nicotine patch group. We do not yet have enough data to report 6-month abstinence rates.

Side effects were noted in 37 patients on combination therapy (54%), 28 patients taking bupropion (38%), and 4 patients taking nicotine patch alone (17%). The most commonly reported side effect was dry mouth (30%), followed by insomnia (19%), and abnormal dreams (11%). 19% of patients who started on combination therapy switched to another treatment compared to 16% of patients started on bupropion alone .

These preliminary results suggest that most patients referred to the SCC are able to take these medications. While side effects appear to be more common with combination therapy, there does not appear to be a difference in the rate of switching treatments. We will be assessing whether the success rate at 2 months and 6 months is significantly different between the groups. We will also be assessing further how well patients tolerate these two regimens. These data will help us determine how the 2 regimens compare in actual practice.

POSTER SESSIONS
Session F: Community and Clinical Interventions

F7
Dissemination of a smoking cessation intervention for hospitalized patients

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Intervening with patients during their hospitalization is an especially effective strategy for increasing smoking cessation rates. Providing the program during hospitalization capitalizes on a teachable moment because smokers are often motivated by illness to quit, they are removed from daily cues to smoke, and many will have undergone the worst withdrawal during hospitalization (Orleans & Ockene, 1993). Despite practice guidelines that call for the identification and treatment of all smokers seen in health care settings, many hospitals fail to offer smoking cessation interventions on a routine basis. This poster presentation will report on an ongoing TRDRP project disseminating a well-validated smoking intervention to seven San Francisco Bay-area hospitals. Dissemination is being conducted in a Veterans health care facility, a county hospital, a University teaching hospital, three community hospitals and a large HMO. Staff involved includes physicians, nurses, respiratory therapists, social workers, psychologists, chaplains, students, and volunteers. Each hospital has developed a unique model for implementing the program and dealing with changes. Barriers to dissemination include thus far identified include: (1) the lack of designated staff members to provide the program on an ongoing basis, (2) variability in implementation of and training in electronic identification of smokers at admission, (3) difficulty implementing the program consistently, given the demands of caring for acutely ill patients and the heavy workload characteristic of health care providers, and (4) competition for time and resources and lack of designated funds. Some of the innovations developed by participating hospitals include: (1) a coordinated program to bridge the gap between inpatient interventions and outpatient follow-up by utilizing public health department personnel, (2) integrated interventions for Spanish and Vietnamese populations, (3) provision of the program by lay volunteers, and (4) integrated "campaigns" using posters, badges, medical staff mailings and closed-circuit television. Strong support of hospital administration is a key to successful implementation. Once implementation has been accomplished and data on effectiveness ascertained, hospitals will move to institutionalize the programs. Preliminary findings indicate that mandates from JCAHO, NCQA, and the AMA to include hospital-based smoking interventions will be critical to ensuring the successful, broader dissemination of these programs into clinical practice.

F8
Pager-cued therapeutic messages for outpatient smoking cessation in a veteran population: a pilot study

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In an effort to explore ways in which technology, such as computers and pagers, can be used to help smokers quit, we are conducting a pilot study to assess the utility and effectiveness of pager-cued therapeutic messages in a group of outpatient veterans. Veterans smoke at approximately double the rate of California's population at large. Many are long-term, heavy smokers with multiple previous quit attempts who are highly motivated to try new tools to help them quit. This pilot study provides participants with personal pagers that are used to receive individually tailored messages to assist and support them as they progress through the cessation process. Up to 60 participants will be enrolled by December 2000.

Patients who express interest in using the pagers to help them quit initially receive brief individual counseling consisting of motivation enhancement, coping skills training, problem-solving, and relapse prevention. After setting a quit date, patients work together with the counselor to select a list of pre-programmed therapeutic messages. These messages are generally constructed to focus on motivation for quitting ("Remember your personal reasons for wanting to quit") or strategies to cope with cravings ("Take a few deep breaths, drink water, etc.") and are delivered at regular intervals during the week, usually twice per day.

During the initial period of this pilot study, our efforts have been directed toward the hiring and training of staff, activation of the pagers and hardware to transmit messages, refining the data collection instruments, construction of the data base, and recruitment of participants. Data are available for the first 21 enrolled participants. Among these, 20 are male, with an average age of 53.9 years, average pack year history of 42.2, an average of 3 prior quit attempts, and currently smoking an average of about a pack of cigarettes (19.9) per day. Stated history of alcohol or drug abuse is prevalent (66.7%), as is history of depression (43.5%). The average baseline score for the Fagerstrom Nicotine Tolerance Test is 3.7 (out of a possible 7) points.

Follow-up data collection will begin at the end of October, 2000. Smoking cessation among participants will be biochemically validated using saliva cotinine assays for those who report abstinence at 6 months. We will also evaluate level of adherence, frequency of use, and degree of satisfaction with the pager-cued messages through the use of self-monitoring diaries, self-report questionnaires, and telephone interviews. This information can be used to further develop ways in which technology can be combined with behavior-change strategies to promote to maintenance of self-regulatory coping responses and relapse prevention.

F9

Motivational interviewing to prevent postpartum relapse: biochemical validation of self-reported abstinence.

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Maternal smoking is a significant threat to fetal, infant, and child health. Pregnancy motivates many women to quit, but postpartum relapse rates average between 50 and 80% in the first year after delivery. There are no published reports of an intervention that prevents women who quit smoking during pregnancy from relapsing after delivery. To address this problem, we are conducting a randomized controlled trial testing the effectiveness of a telephone-based motivational counseling program to prevent postpartum relapse.

Subjects were recruited from the diverse prenatal population of a large multispecialty group-based managed care organization. We enrolled 270 currently abstinent women in their 8th month of pregnancy who had reported at the start of prenatal care that they had quit smoking in response to pregnancy. Subjects agreed to participate in baseline and follow-up telephone interviews and to provide a saliva sample at follow-up to screen for cotinine, a metabolite of nicotine.

To date, we have completed 6-month follow up with 217 subjects. Prior to commencing the follow-up interview, subjects were reminded about the biochemical verification of smoking status with a saliva sample. More than two-thirds (69.2%) reported abstinence. We mailed saliva collection kits to all women reporting abstinence. Three-fourths of self-reported abstainers returned their kits and 25% did not. On follow up, few (n=3) women refused to return the kit. Most said they would return the kit, but didn't. Others said they had returned the kit, but it was never received.

We compared women who did and didn't return their kits on characteristics that have been identified in the literature as predictors of postpartum relapse. While we didn't find baseline differences based on sociodemographics, we found that women who reported they had gained too much weight were less likely to return their kits (59.2% v. 85.1%, p=.001), as were women with many smokers in their social network (50% v. 78.5%, p=.01). Similarly, women who before pregnancy smoked their first cigarette of the day within 30 minutes of waking were less likely to return their kit (62.1% v. 78.7%, p=.07) and women who planned to bottle feed their infants compared with those who intended to breast feed only were also less likely (67.3% v. 80.9%, p=.08). Finally, women who failed to return their kits had reported less confidence in their ability to maintain postpartum abstinence (mean of 8.5 v. 9.2 on 1-10 scale, p=.05). By convention, self-reports of nonsmoking that are not confirmed biochemically are classified as smoking. Our comparison of self-reported abstainers who did or didn't return their saliva cotinine collection kit supports this practice.

Results of the randomized trial testing the effectiveness of a postpartum relapse prevention program will be available in early 2001. Despite the health hazards, 25% of US women between the ages of 18 and 44 continue to smoke. More than a million children in California are exposed to environmental smoke in their homes. If effective, this relatively low-cost intervention could make an important contribution to the health of young women and their families.

F10

Transdermal nicotine replacement for hospitalized smokers: a randomized trial

Simon, Joel A.
University of California, San Francisco / San Francisco VA Medical Center

BACKGROUND: There are at least 6.5 million adult smokers hospitalized yearly in the US, providing doctors and health educators a window of opportunity to reach smokers with smoking cessation interventions. Patients are often hospitalized for a smoking-related illness, may not be able to smoke while hospitalized because of restrictive smoking policies, and may, therefore, be more open to quitting smoking. To date, relatively few controlled clinical trials have examined whether hospital-based smoking cessation interventions are effective in helping smokers quit. Because the effectiveness of the nicotine patch for in-patient smoking cessation has not been studied previously, we are conducting a clinical trial in which study participants will begin use of the nicotine patch during their hospitalization.

METHODS: Patients who are current cigarette smokers and who are hospitalized for at least 48 hours are being randomized to two groups - an intervention group and a minimal-contact comparison group. Patients in both groups receive the nicotine patch for 2 months. The intervention group is given face-to-face advice on practical smoking cessation strategies by a counselor, self-help booklets and is shown a 10 minute stop-smoking videotape prior to hospital discharge. The intervention group is followed up by phone weekly for one month and then monthly for 3 months. The minimal-contact comparison group is given the self-help booklet only. All patients are being followed by the study personnel during the 12 months following hospital discharge to determine rates of tobacco abstinence at 6 and 12 months. To confirm tobacco cessation histories biochemically, cotinine levels are being obtained at one year in all those study subjects who report smoking abstinence.

RESULTS: A total of 223 current smokers admitted to the San Francisco VA Medical Center between October 1997 and March 2000 have been enrolled. Six-month follow-up data are available for 96 intervention participants and 99 comparison participants. A total of 34% of the intervention participants (n = 33) and 23% of the comparison participants (n = 23) have quit smoking by self-report (p = 0.09). One-year self-report follow-up data are available for 70 intervention participants and 69 comparison participants. A total of 37% of the intervention participants (n = 26) and 19% of the comparison participants (n = 13) have quit smoking by self-report (p = 0.02). Thus far, we have confirmed smoking cessation by saliva cotinine or spousal/family proxy for 13 of the intervention quitters and 6 of the comparison quitters (p = 0.11). There have been 13 deaths, 5 among the intervention group and 8 among the comparison group.

CONCLUSIONS: By self-report, smokers enrolled in an intensive hospital-based smoking cessation intervention that includes the nicotine patch are more likely to have quit smoking at 6 and 12 months. If the self-reported quit rates are confirmed by biochemical validation and proxy, our findings would suggest that hospitalized smokers should be offered the opportunity to participate in intensive hospital-based programs to increase smoking cessation rates after hospital discharge.

F11

Bupropion for smoking cessation: a randomized trial

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BACKGROUND: Standard smoking cessation interventions include counseling, nicotine replacement therapy, and self-help literature. Because smokers are more likely to have a past history of depression and smoking cessation may increase symptoms of depression, the use of antidepressant medication for smoking cessation has been proposed as a possible aid for smoking cessation. A few studies have reported bupropion, an antidepressant, an effective adjunct for smoking cessation. However, the effectiveness of bupropion has been studied in only a few populations to date.

METHODS: We are conducting a randomized blinded clinical trial at the San Francisco VA Medical Center in which approximately 360 smokers will receive standard treatment that includes 2 months of the nicotine patch, counseling, and use of self-help literature. In addition, approximately 50% of participants will be randomly assigned to receive 7 weeks of bupropion whereas the remaining 50% of participants will be randomly assigned to receive an identical placebo. Neither the participants nor the investigators will know who has been assigned to receive the active drug. If the addition of bupropion to standard therapy increases biochemically-confirmed smoking cessation rates at one year, it may become an important addition to smoking cessation programs targeted at diverse populations of heavily addicted smokers.

RESULTS: This study is currently recruiting participants. A total of 215 current out-patient smokers, both veteran and non-veterans, have been enrolled to date. Six-month follow-up data are available for 173 participants. By self-report, 57% of the participants (n = 98) have quit smoking at the end of treatment (8 weeks) and 39% of the participants (n = 67) have quit smoking at 6 months of follow-up. A total of 118 participants have been followed for 12 months. The validated quit rates for these participants are 19% of the bupropion group and 26% of the comparison group ($p = 0.35$) A total of 3 participants have died, two in the bupropion group and one in the comparison group.

CONCLUSIONS: If the current trend continues, the use of bupropion together with nicotine replacement and counseling would not appear to further increase quit rates over that expected from nicotine replacement and counseling alone.

Session G: Environmental Tobacco Smoke

G1

Implementation of California AB13: Smoking Ban in Bars

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In 1995 California AB13, prohibiting smoking in enclosed workplaces, took effect. In 1998 the smoking ban was extended to bars. We are studying the implementation and enforcement of the smoking ban in bars by determining the variability in compliance among bar employers; exploring enforcement at the local level; and describing activist efforts to support implementation. We are interviewing three sets of respondents: a random sample of bar owners (to date N=60); enforcement officials (to date N=20); and tobacco control activists (to date N=11). Transcriptions of interviews are content analyzed and coded in an ongoing, iterative process to capture recurrent themes.

When the workplace smoking ban went into effect in 1995, most employers voluntarily complied. The bar owners we interviewed claimed they were in compliance, but with further discussion it became clear that they did not mean 100% compliance 100% of the time. Often they made exceptions, such as letting patrons smoke after 10 p.m., during special events, or in designated areas of the bar. The consequences of non-compliance were minimal: fines were relatively low and enforcement lax.

Many localities had not been faced with the task of having to enforce this state law until it was extended to bars. Local government often lacked resources for enforcement. Enforcement efforts were slow to be initiated, and typically over time the responsibility for enforcement was passed from one local agency to another.

This resulted in an important role for tobacco control activists from private non-profits such as ACS, AHA, and ALA. These voluntary organizations generated and maintained visibility for, and public support of, the law. Volunteers intervened and repaired breakdowns in the enforcement process, *i.e.*: initiating a *writ of mandamus* against a city for failure to enforce; educating and counseling district attorneys and judges to ensure their follow-through in prosecuting labor code violators; encouraging local district attorneys to file unfair business practices charges against non-compliant bars; and case finding to recruit individuals with disabilities to for test cases to ensure access of people with ETS sensitivities to public accommodations (such as bars). In contrast, those who worked for Proposition 99-funded programs are prohibited from engaging in enforcement activities, therefore, were limited to using health education approaches that did not appear to be as effective in facilitating compliance.

Reduction in exposure to ETS and the consequent reduction in cancer and heart disease will only be realized if Labor Code 6404.5 is successfully implemented. This study has implications for future legislation in terms of: **Compliance** : getting a law passed does not necessarily mean that it will be complied with. Health education models may not work with intransigent entrepreneurs such as bar owners. Activists should address bar owners' fear that compliance will threaten profit, and raise their awareness of possible financial liability from workers' compensation suits on ETS exposure.

Enforcement: The AB13 experience demonstrates a need for tobacco control legislation to clearly delegate and delineate enforcement mechanisms. Even though local control of enforcement appeals to tobacco control activists because of their history of success at the local level, not every locality will support enforcement.

G2

Bars and other Environmental Contexts as Predictors of Approval for the Smokefree Bars Law

Friis, Robert H.
California State University, Long Beach

Project Goal: The 1998 California Smokefree-Bars Law was designed to protect California workers in bars from secondhand smoke. The major goal of our research project is to determine the response to the Smokefree-Bars Law from Long Beach, the fifth largest city in California and one of three cities with its own Health Department. To achieve that goal we are gathering results in five areas: compliance with the law, business data, law-related attitudes of bar personnel, community approval for the law, and print media coverage of the law.

Study Objective : Results for this study were drawn from the community's approval for the law and can be applied to enforcement issues. While the law is statewide, local jurisdictions are responsible for the law's enforcement. This study analyzed approval of the law as a function of frequency of going to bars and other locations in order to contribute to the City's evolving, enforcement protocol.

Methods: Data were collected from a random sample of 1502 residents of the city of Long Beach, who were at least 18 years old and agreed to participate in a telephone survey during the last two months of 1998. Response rate was 76%. Approval for the law among bargoers was compared with those who stayed home, had friends over, went out to dinner, went to parties, and hung around with friends in public places, while controlling for smoking status and anti-tobacco attitudes and beliefs.

Results: Among the entire sample, 66% approved of the Smokefree Bars Law. However, the more often people went to bars, the less likely they were to approve of the law: among those who went to bars at least once a week, 49% approved of the law. When frequencies of going to all five locations were studied as potential predictors of approval — while controlling for smoking status — going to bars was an independent, negative predictor of approval, and going to restaurants and hanging out with friends in public places were independent, positive predictors of approval. However, when five tobacco attitude and belief factors were added, all five factors were significant, but bar-going was no longer related to approval.

Future Directions and Conclusions : By the end of the year, another community survey will be conducted, and we will determine any changes in approval for the law since the law's implementation. Based upon these 1998 survey results, the more often people went to bars, the less likely they were to approve of the Smoke-free Bars Law. Those concerned with compliance and enforcement could note, however, that even among those who went to bars most frequently, nearly 50% approved of the law. Moreover, when anti-tobacco and secondhand-smoke beliefs were considered along with these environmental contexts, bar-going was no longer a significant, negative predictor of approval. Therefore, health promotion interventions that address tobacco attitude and belief factors should help boost approval for the Smokefree-Bars Law, as well as with other tobacco-control laws and ordinances, which can lead to increased compliance, decreased secondhand-smoke exposure, and improved health.

POSTER SESSIONS
Session G: Environmental Tobacco Smoke

G3
Compliance with the Smokefree-Bars Law in Long Beach from 1998 to 2000

Friis, Robert H.
California State University, Long Beach

Project Goal: A 1995 law designed to protect California workers from secondhand smoke was extended to bars and implemented January 1, 1998. Our major research goal is to determine the response to this Smokefree Bars Law from the City of Long Beach, the fifth largest city in California and one of three with its own Health Department. To reach that goal we have been collecting and analyzing results in five areas: compliance with the law; business data; attitudes of bar personnel toward tobacco and the law; approval for the law within the community; and print media coverage of the law.

Objective: In this study we drew on measures of compliance gathered from 1998 to 2000. Our aim was to observe trends over time in compliance, determined from on-site observations at bars, numbers of complaints telephoned to the Health Department, and also from print media coverage of the law.

Methods: Observations of compliance with the Smokefree-Bars Law were made at 40, City of Long Beach bars that had permitted smoking before the law. Daytime observations of smoking and the presence of ashtrays inside and outside the bars were gathered during Fall 1998; Spring 1999; Fall 1999; and Spring 2000. During Spring 2000, we added measures of cigarette odor inside the bars and the presence of cigarette butts and containers outside the bars, along with additional observations during early-evening hours, after 5:00 p.m. Numbers of telephoned complaints of law violations were provided by the Health Department Tobacco Education Program. For print media coverage we relied on opinions published in California travel guides.

Results: According to both onsite observations and numbers of telephoned complaints, compliance with the ban on smoking in bars has increased over time. In Fall 1998, smoking was observed in more than 30% of the bars in contrast to about 15% of bars by Spring 2000. Compliance was consistently higher for restaurant-bars compared to stand-alone bars. During Spring 2000, odor of cigarette smoke was significantly lower within restaurant-bars than in stand-alone bars during both the day and early evening hours. Compared to daytime, compliance during the early evening was lower, and the proportion of bars with people smoking outside was greater. Travel guides referred to early, militant wishes to repeal the law, variations in compliance by type of bar, to nonsmokers appreciating not going home smelling like an ash tray, the judgement that most bars comply, and more recently to people smoking outside, where cigarette butts are littered.

G4
Arguments Against Workplace Smoking Restrictions: Case Studies of Two Regulations

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University of California, San Francisco

The purpose of this study is to provide information to policy makers and health advocates on effective ways to use empirical research in the formation of tobacco control policies. We compared the regulatory policy development in two states and the extent to which policy makers utilized research findings when developing indoor air regulations.

We conducted content analysis and qualitative coding of written commentaries and hearings submitted for the Maryland Indoor Air regulation and the Washington Clean Indoor Air Act. We coded each submission for position, affiliation, and arguments used. We also coded references cited by type and quality of journal article. Transcripts and written commentaries were analyzed together.

In Maryland, 56% (157/345) of the comments were in opposition to the regulation. In contrast, in Washington only 27% (52/193) of comments were against the regulation. In both states, opposition to the workplace smoking regulations came primarily from the tobacco industry and small businesses, and appeared to be coordinated at a national level. Although scientific arguments were used less frequently than other types, arguments about science were used more often by those opposed to the regulations than by those in favor ($p = .0002$, Chi2, in Washington, and $p = .8197$, Chi2, in Maryland). Arguments about how science should be evaluated occurred frequently (45% of the Washington commentary, and 27% of the Maryland commentary) and differed between commentary supporting or opposing the regulation. Supporters emphasized the quantity of the evidence, while opponents produced abundant critiques of the reliability, validity and quality of the evidence on which the regulations were based. Non-scientific arguments, such as ideological, economic, building management, political, and procedural, were mentioned more often in Washington (98% of comments) than in Maryland (78%).

We assessed the type of the references and quality of journal articles that were submitted with the comments for both states. Those opposing the regulations submitted more documents than those in favor in both Maryland (68%, 350/515) and Washington (99%, 592/596). However, 21% of the references in Maryland were from non-peer reviewed publications such as conference presentations and symposia compared to 2% in favor (we excluded Washington from further analysis due to the low number of articles submitted in favor, n=2). We further analyzed journal article quality in Maryland. In Maryland, journal articles cited in support of and against the regulation had similar median years of publication (1990 vs. 1989) and percentages that were peer reviewed (89% vs. 85%). However, references cited by those against the regulation had a lower mean impact factor (1.7) than those cited in support of the regulation (2.8) ($p = .0006$).

Our papers are currently under peer review.

Discussions of health effects and economic research play a substantive role in smoking restriction regulatory policy development. By inundating regulatory officials with research of questionable credibility and quality, the tobacco industry attempts to slow the regulatory process. Our findings suggest the need for tobacco control advocates to counter these tactics by keeping the debate focused on the adverse health effects of passive smoking by citing the strong body of literature in this area.

G5

California environmental tobacco smoke risk assessment

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We studied the extent to which empirical research findings were used to develop the Environmental Protection Agency of California risk assessment of environmental tobacco smoke. The risk assessment was a collaborative effort by the Office of Environmental Health Hazard Assessment (OEHHA) and the Air Resources Board (ARB) to do a comprehensive review of scientific data with collected public input. Our data consisted of public testimony at the workshops, as well as public commentary and citations submitted during the five years (1992-1997) of the risk assessment process. We examined the position, affiliation and arguments presented in the public commentary, as well as OEHHA's response to the public commentary and if it changed the risk assessment. We also examined the publication type and quality of journal articles cited. We focused our analysis on the commentaries that referred to the risk assessment chapters on respiratory health effects, carcinogenic effects and cardiovascular health effects.

The critics were overwhelmingly represented by tobacco industry affiliates (30/36, 83%), whereas the supporters tended to be affiliated with government (3/8, 38%), health professional (2/8, 25%) and nonsmokers' lay organizations (1/8, 13%) or private citizens (2/8, 25%) (p=.0001). Both sides used scientific arguments more then any other argument. Critics were more likely to use arguments about methods (22/23, 96%) than supporters (4/6, 67%) (p=.04). Of these methods arguments, comments about bias and confounders were the most frequent (21/23, 91%). More then half of the critics also argued that appropriate procedures for conducting risk assessments were not followed (13/23, 57%). In contrast, supporters did not focus on any one specific argument. Of submitted commentary, 16% resulted in a change to the risk assessment. Journal articles were the most frequently cited type of reference by both supporters (39/60, 65%) and critics (1022/1526, 67%). However, journal articles submitted by the critics had lower impact factors than those cited by supporters (2.6 vs. 3.5, p=.03).

We are currently in the process of writing the results to be submitted for publication. We want to convey this information to public health advocates as well as scientific agencies and law-making bodies to assist them in countering tobacco industry tactics concerning the public health and environmental tobacco smoke.

Risk assessments are important in reducing both human and economic cost of tobacco use. Our previous work has shown that government reports, such as the United States EPA risk assessment of passive smoking, are heavily relied upon to support smoking restrictions. However since this risk assessment was "vacated" by the North Carolina courts in July 1998 on procedural grounds, the California risk assessment may now play a more important role in future smoking restriction debates. Also, analysis of the tobacco industry's strategies could enable public health advocates to anticipate and prevent successful legal action by the tobacco industry against the California risk assessment. Furthermore, the California risk assessment was the first to include a section on the cardiac effects of passive smoking, thus making this risk assessment an important case study to support current and future smoking policies.

G6

Exposure to environmental tobacco smoke in pregnant women: the association between self-report and serum cotinine

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The risk of delivering a low birthweight infant as the result of exposing a nonsmoking pregnant woman to environmental tobacco smoke (ETS) is not well defined. The method of ascertaining ETS exposure during pregnancy may explain the lack of consistent study findings. In a large sample of pregnant women, we compared distributions between two methods of ETS exposure: self-report and cotinine, a nicotine metabolite, from serum. At delivery, subjects were asked about duration and location of exposure to ETS during their 4th and 5th month of pregnancy. A single cotinine measurement was assayed from serum collected at 15-19 weeks gestation (limit of detection = 0.05 ng/mL). Self-reported (hours per day) ETS exposure was correlated (r=0.38) with serum cotinine concentration. Regression analysis revealed that self-reported hours per day (as a cubic polynomial and as categorical) was a significant predictor of (log) serum cotinine. While 72% of subjects reported no exposure to ETS, almost all of this subgroup had measurable levels of cotinine in serum. Therefore, studies of pregnant women based upon an hours per day ETS exposure question have in all likelihood misclassified a sizable portion of ETS exposed women as "unexposed." If ETS results in lower birthweight babies, then these studies have underestimated this effect.

POSTER SESSIONS
Session G: Environmental Tobacco Smoke

G7

Association Between Exposure to Environmental Tobacco Smoke and Self-reported Health Conditions: The Northern California Kaiser Permanente Experience, 1979 to 1985.

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Northern California Kaiser Permanente

We examined the cross-sectional association between exposure to ETS and self-reported health conditions in a large population-based sample. Data were collected at a health maintenance organization in Northern California using written questionnaires between 1979 and 1985; the sample included 41,258 persons (38% men, 62% women) aged 15-105 years (mean ± SD, 39 ± 15 years) who reported never using any tobacco product (cigarettes, cigars or pipe). Sixty-seven percent of men and 64 percent of women reported any ETS exposure (sum of ETS exposure at home, in small spaces other than home, or in large indoor areas) greater than 1 hour per week; 13 percent of men and 17 percent of women reported a total duration of ETS exposure = 40 hours per week, respectively.

ETS exposure = 40 hours per week (compared to exposure to ETS of 1 hour per week or less and after adjusting for age, sex, race, education level, marital status, alcohol consumption, physical activity at work, total cholesterol, body mass index, hypertension, diabetes and occupational hazards as appropriate) was independently and positively associated with self-reported heart disease (RR=1.15; 95% CI=1.06-1.26), intermittent claudication (RR=1.17; 95% CI=1.02-1.35), chronic cough (RR=1.30; 95% CI=1.10-1.54), hay fever\asthma (RR=1.23; 95% CI=1.13-1.33), hearing loss (RR=1.28; 95% CI=1.08-1.53), severe headaches\migraine (RR=1.43; 95% CI=1.32-1.55), and to having, on average, flu/cold symptoms for more than 10 days per year (RR=1.80; 95% CI=1.64-1.97). No significant associations were found for emphysema of the lungs, stroke, or overall cancer or tumor (no information existed on site-specific cancers). The relative importance of ETS exposure at home, in small spaces other than home, or in large indoor areas was also investigated.

These results suggest and independent association between exposure to ETS and ill-health. Prospective analysis of outcomes is required to substantiate these findings.

G8

Prevalence and patterns of reported environmental tobacco exposures in the California Teachers Study cohort.

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California Department of Health Services / Public Health Institute

This study is a descriptive analysis of the prevalence and patterns of environmental tobacco exposures (ETS) in a large, well-defined cohort of California women. Extensive data on lifetime ETS exposures were derived from self-administered surveys completed by members of the California Teachers Study (CTS) cohort. Established in 1995 to study risk factors for breast cancer, the CTS cohort is comprised of over 133,000 active and retired female school teachers and administrators. Cohort members are re-contacted annually and asked to complete additional surveys on a biannual schedule. Data for this analysis are derived from the 99,527 women who responded to the second survey, which included detailed questions on ETS exposures. Data from this survey offer extensive lifetimes histories of ETS exposure including the intensity and duration of exposures during childhood and each subsequent decade of life for exposures in the home, at work and in other settings. The prevalence of reported ETS exposures were compared to other population estimates for California women. Tobacco exposures within this cohort are somewhat unique in that only 5% of cohort members were current smokers at baseline and nearly two-thirds were lifetime non-smokers. Among the lifetime non-smokers, more than two-thirds reported exposure to household sources of ETS, half reported workplace ETS exposures and over a third reported ETS exposures in other settings. By combining the information on age-period specific exposures with birth year information, temporal patterns of exposure also were explored.

This descriptive analysis is part of a larger effort to conduct an integrated evaluation of both the patterns and correlates of ETS exposures with a detailed analysis of how ETS relates to a variety of chronic disease outcomes of concern, particularly common cancers and acute asthma events. In order to allow sufficient follow-up time, the outcome analyses for this project are scheduled for next year.

Understanding the patterns and correlates of ETS exposures is critical in planning targeted public health interventions. Establishing baseline exposure profiles is necessary to evaluate future initiatives to reduce ETS exposures. Furthermore, the extensive information that the CTS cohort provides on lifetime ETS exposures, coupled with the large size of the cohort, offers the unique opportunity to comprehensively evaluate the role of ETS exposures in selected disease outcomes in a way that no other large-scale study to date has been able to do.

G9

Evaluation of office ETS exposures in relation to AB13

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The overall goal of this research is to investigate the effectiveness of smoking rooms in preventing exposures of occupants of adjoining offices to environmental tobacco smoke (ETS). The objectives of the first phase of the study include evaluating the effects of smoking room design features and modes of operation on the leakage of air and ETS from the smoking room to the surrounding interior space. This research will result in information to guide improvements in the design and operation of smoking rooms.

A series of laboratory experiments are underway at Lawrence Berkeley National Laboratory (LBNL) to quantify the rate of ETS leakage from a smoking room to a non-smoking area as a function of the physical characteristics (such as ventilation system design and operation) and ways of using the smoking room (for example, frequency of door opening). A mathematical model of ETS leakage will be developed and compared to measured ETS leakage. The performance of the model will also be tested with data collected in several California office buildings.

Our project requires accurate real-time quantitation of ETS, as well as discrimination of dilute ETS from other sources of particles, especially in the non-smoking areas. In order to choose the most appropriate equipment for the study, we used LBNL's environmental chamber to compare the sensitivity and selectivity of three real-time instruments for ETS, in the presence of ambient particulate matter (PM), and ambient PM mixed with diluted diesel exhaust, woodsmoke or meat smoke. Simultaneous real-time monitoring was conducted with a quartz crystal microbalance impactor (QCM), a polycyclic aromatic hydrocarbon (PAH) monitor, and a dual wavelength aethalometer that monitors light-absorbing particles with both visible and ultraviolet light. Integrated particulate mass concentrations were determined by filter gravimetry.

ETS was maintained at 500 $\mu g\ m^3$ in the 15 m^3 smoking room, while it was ventilated at 7 air exchanges per hour (ACH). An adjacent room (19 m^3) was ventilated with unfiltered ambient air at one ACH. A fraction of the exhaust from each combustion source entered the supply duct for the non-smoking room, along with ambient air. Infiltration of ETS into the non-smoking room was controlled by adjusting the pressure difference between the two rooms.

Each instrument had strengths and drawbacks. All three devices had more than sufficient sensitivity to quantitate the infiltrating ETS, but they differed significantly in their ability to discriminate ETS from the other sources. The QCM reported particle mass size distributions that peaked at smaller diameters for sources than for ambient particulate matter. However, based on size alone, ETS could not be distinguished from the other sources. Interpretation of the data from the PAH monitor required knowledge of the instrument's response function for each type of source. The dual-wavelength aethalometer did successfully discriminate ETS from the other PM sources, but it recorded somewhat noisier signals than the other instruments. Both ETS and wood smoke absorbed much more strongly in the ultraviolet than ambient particulate matter, diesel exhaust and meat smoke. ETS was 2 to 3 times more sensitive to ultraviolet light than wood smoke was. We chose the dual-wavelength aethalometer for the laboratory and field phases of our study because it quantitated ETS in the presence of ambient particulate matter and the other combustion sources, without reference to supplementary information from other instruments.

G10

Vapor-Phase Organics in ETS: Dynamics and Exposure

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Environmental tobacco smoke (ETS) is the complex mixture of chemical vapors and particles to which non-smokers are exposed when in a room or house with smokers. ETS exposure is associated with increased risk of lung cancer, heart disease, and childhood asthma, but great uncertainty remains about which ETS components are most responsible for adverse health effects. The relative and absolute room air concentrations of individual ETS components are influenced by smoking frequency, ventilation, and the tendency of some less volatile gases to stick (sorb) to indoor surfaces. Sorbing compounds are selectively removed from the air during smoking – thus reducing exposure – but can later desorb from surfaces, resulting in exposure long after active smoking has ceased. Significant ETS exposure can also occur if non-smokers enter a room shortly after smoking has occurred.

The overall objective of this project is to further characterize ETS exposure under realistic home conditions. We first conducted a series of experiments to quantify "effective" emission rates of 28 organic vapor ETS components under a wide range of smoking rates, ventilation, and furnishing levels. These "effective" rates include sorption losses and can be used to predict exposure when combined with simple indoor environment models. Results indicate that tobacco-specific pyridine and 3-ethenylpyridine (3-EP) are emitted in relatively constant proportions to many individual vapor components of ETS, even as conditions change. These experiments also allow us to identify ETS components for which sorption is an important removal mechanism that must be considered in more detailed modeling of ETS emissions and exposures. Phenol and naphthalene, ETS components specifically identified by the state and federal authorities as toxic air pollutants, both fall into this category. A second series of experiments aims to quantify the effect, on exposure, of long-term sorption and re-emission of semi-volatile ETS components. This framework includes several weeks of daily smoking in a furnished 50-m^3 chamber, and pollutant concentration measurements during 4-hr smoking, 8-hr post-smoking, and 12-hr "background" periods. The third axis of investigation focuses on the development of semi-empirical models to characterize sorption losses, and potentially re-emission, of ETS components in typical indoor settings.

This work fills an important gap in the literature, since previously reported ETS emission factors have been measured almost exclusively in unventilated stainless steel chambers. The emission ratios that we report can be combined with measured pyridine and 3-EP exposure distributions to estimate exposure to ETS-generated toxics under a wide range of real world conditions. The daily smoking experiments will provide unique data to assess the longer-term implications of sorption-desorption processes on exposure to ETS, and other indoor air pollutants. Overall, this work will help toxicologists focus on the most important disease-causing agents in ETS and will provide guidance for epidemiologists to better assess ETS exposure based on household smoking and ventilation rates.

POSTER SESSIONS
Session G: Environmental Tobacco Smoke

G11
Quantification of population exposure to secondhand smoke

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Environmental Tobacco Smoke (ETS) is a complex mixture of toxic pollutants, including many carcinogens. Although much is known about the chemical composition of ETS, relatively little is known about the amount of exposure that people receive in common everyday locations. The goal of our research is to develop new methods to measure, understand, and predict the exposures of Californians to secondhand smoke, with emphasis on common exposure locations such as automobiles and homes. In particular, our research assesses changes in ETS population exposure in response to intervention and public smoking laws.

To measure secondhand smoke in the home, we are developing a new fully automated instrument package that can measure air pollutant levels indoors without an operator, the Continuous Air Monitoring Package (CAMP). The CAMP can run quietly in a home and automatically measure and record the temperature and humidity every minute, as well as several important indicators of secondhand smoke – carbon monoxide, particle-bound polycyclic aromatic hydrocarbons (PAH), and particle size count densities in 14 different sizes. We have conducted 24 controlled experiments in 3 houses and several controlled experiments in 2 automobiles to test and evaluate these real-time monitors by making and recording pollutant measurements every minute. Our experimental studies in houses show that the CAMP can detect and measure accurately the pollutants generated by a single cigarette in a home, and the ETS concentrations measured in an automobile were found to be extremely high.

We are also developing a suite of mathematical models that can predict how the concentrations are affected by the smoking pattern within a home. By predicting indoor concentrations and exposures as a function of smoking activity and home characteristics, our models will facilitate assessment of health risks associated secondhand smoke and identification of steps that can reduce exposure and health risks. For example, closing doors and opening windows in a home affect the air exchange rates and the room-to-room transfer of pollutants, thereby reducing the exposure of the residents. Our models can tell us quantitatively how much these intervention steps will reduce ETS exposure.

To assess levels of secondhand smoke experienced by the California population, we are examining the personal activity patterns from the recorded diaries of 11,800 people that include their reported exposure to secondhand smoke. We developed a computer model that combines activity pattern data with our concentration measurements to produce statistics of California population exposure to ETS. These models allow us to predict changes in population exposure statistics in response to different intervention strategies, such as reducing smoking in motor vehicles, homes, stores, bars, and restaurants. We have measured the concentrations in common exposure locations and are using these measurements as input to our activity pattern-exposure models. The demographic information included with the activity pattern diaries will be used to predict differential effects on human exposure of alternative intervention strategies for different demographic groups such as gender and race.

G12
Alternative Sources of ETS Exposure in Infants

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Inhaling secondhand smoke and ingesting breast milk are the two most common sources of environmental tobacco smoke (ETS) exposure in infants. However, infants may be exposed to secondhand smoke from other sources as well. This study explored the importance of indoor dust and surface contamination as sources of secondhand smoke exposure in infants. Specifically, indoor environments and secondhand smoke exposure were compared in three types of homes: (a) homes in which mothers smoke cigarettes in the presence of their infants (i.e., direct exposure group; DEG), (b) homes in which mothers smoke cigarettes away from their infants (i.e., indirect exposure group; IEG), and (c) homes of non-smoking parents who do not expose their children to ETS (no exposure group; NEG).

Subjects were 45 mothers and their infants (12 months and younger) who were recruited from San Diego County sites of the Women, Infants, and Children Supplemental Food and Nutrition Program and advertisements in community newspapers. Each family was visited three times over the course of one week and multiple urine, hair, dust, surface wipe, and air samples were collected to determine nicotine and cotinine levels. In addition, current smoking behavior, mother-reported ETS exposure, cleaning habits, and personal background were assessed through personal interviews and dairies.

Analyses of nicotine concentration in air, dust, and wipe samples in the living room and the child's bedroom showed significant differences in contamination levels between NEG, IEG, and DEG. Analyses of hair nicotine and cotinine as well as urine cotinine revealed marked differences in exposure levels between the three groups. These findings indicate that nicotine accumulates in living room and bedroom surfaces and in household dust. Smoking away from the child (e.g., outside, in another room) reduced but did not prevent the contamination of household dust and household surfaces or the exposure of infants to ETS. In summary, these findings suggest that indoor dust and surface contamination present two alternative sources of passive exposure of infants to secondhand smoke.

G13

Indoor Measurements of Environmental Tobacco Smoke

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Environmental tobacco smoke (ETS) - the smoke released from the burning end of the cigarette - is one of the most common sources of carcinogens to which the general public is exposed. However, little is known about the size and extent of potential exposures to ETS. The objective of this research project is to improve the basis for estimating ETS exposures in a variety of indoor environments. The research utilizes experiments conducted in both laboratory and 'real-world' buildings to: 1) study the transport of ETS species from room to room; 2) examine the viability of using various chemical tracers as tracers for ETS; and 3) evaluate to what extent re-emission of ETS components from indoor surfaces might add to the ETS exposure estimates.

In previous years we investigated multi-zone transport of ETS in a three-room environmental chamber. One room (simulating a smoker's living room) had been conditioned extensively with ETS, while a corridor and a second room (simulating a child's bedroom) remained smoke-free. At several inter-room transport rate conditions ETS particles were observed to disperse from the smoking room into the other rooms, eventually reaching the same concentration throughout. However nicotine was quickly adsorbed on unconditioned chamber surfaces of the corridor and bedroom, resulting in very low levels, even during smoking episodes. ETS particle tracers: ultraviolet-absorbing particulate matter (UVPM) and fluorescent particulate matter (FPM) characterized the transport of ETS particles into the non-smoking areas much better than did nicotine. Of the particle tracers, UVPM was found to be the most sensitive and chemically stable. Solanesol, an ETS-specific tracer, was measured but proved to be too unstable to be a reliable marker of ETS.

These results suggested that because of nicotine interactions with the building materials, ETS exposures inferred from nicotine measurements might often be either underestimated or overestimated. Nicotine used to estimate a child's bedroom ETS exposure from smoking occurring in the living room may underestimate the child's particle-component of exposure. Similar measurements made in the smoker's living room during non-smoking periods may be elevated due to re-emission of nicotine from surfaces, leading to a possible overestimate of ETS particle exposures during time spent in the living room.

This year we conducted a pilot field study to verify the chamber findings in real environments. We studied three single-smoker residences during five one-week periods while each household's smoker was enrolled in a six-week smoking cessation program. Week-long measurements in the real homes showed that UVPM is an effective ETS tracer in both smoking and non-smoking rooms and that UVPM has more than sufficient sensitivity for residential exposure assessment. Nicotine traced ETS well in the smoking areas of all three houses, but was inconsistent in the non-smoking rooms where surfaces were presumably not saturated with nicotine. These results indicate that in areas with unknown smoking histories nicotine will not trace ETS reliably. Limited data suggest that sorption and/or re-emission phenomena may have contributed background nicotine of ~0.8 mg m^{-3} to the non-smoking area of the home with highest smoking rate. The other homes showed no background nicotine in smoking and non-smoking rooms. The pilot field study did not uncover significant bias from other particle sources during spring weather in residential neighborhoods. Evaluation of the selectivity of UVPM for ETS compared to other particle sources requires supplementary measurements of ETS in the presence of wood smoke and diesel exhaust (see Poster 8RT-0157). Based on the pilot study, indoor fine particle mass and UVPM (both adjusted for infiltrating outdoor particles) correlated well with ETS emission rates (calculated from cigarette butt counts and ventilation measurements using a mass-balance model). Results from FPM analyses were similar to those from FPM but slightly more variable, while solanesol was found to be unreliable for one-week measurements. We tracked ETS reliably even in non-smoking rooms after smokers reduced their smoking rate by 50 to 100% during the six-week program.

Session H: Cardiovascular & Reproductive Health Effects

H1

Maternal DNA repair of tobacco-induced sperm lesions

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Epidemiological evidence is accumulating that father's cigarette smoking is associated with reduced semen quality, as well as DNA damage in sperm, spontaneous abortion, malformation, neonatal death, and childhood cancer. The purpose of this research is to generate new knowledge about the molecular and genetic factors in paternal germ cells and zygotes that can increase the risk for abnormal pregnancies in couples where the father smokes. We hypothesized that: (a) paternal exposure to tobacco smoke induces DNA lesions in sperm that accumulate during the repair-deficient period of spermatogenesis and are converted into chromosomal aberrations after fertilization; and (b) deficiencies in DNA repair genes of the female partner can increase the frequency of paternally transmitted chromosomal defects.

We have selected the mouse as the model species because the genetics of the mouse are well understood and there is a high degree of similarity between mouse and human DNA repair genes and the role of maternal DNA repair function in processing sperm damage. We determined whether DNA lesions accumulate in sperm after chronic exposure to diepoxybutane (DEB), a component of tobacco smoke. Male mice were treated with daily doses of DEB for up to three weeks and then mated with untreated females. Our data suggest that the last week before fertilization is the critical time-window for the induction of chromosomal damage in sperm after exposure to DEB. Comparisons with acute exposure studies suggest that genetic lesions do accumulate in sperm.

We also conducted experiments to examine specific maternal DNA repair functions in the processing of DNA sperm damage. We determined the levels of gene transripts for p53 (a cell cycle control gene), XPA and XPC (genes involved in the recognition of DNA adducts), Ku86 and Rad54 (genes involved in the repair of DNA double strand breaks), XRCC1 (a gene involved in the repair of damaged DNA bases) and Ape1 (a gene involved in the recognition of abasic sites) in unfertilized and fertilized eggs using RT-PCR. We have found differences in the expression pattern of these genes. RAD54 was consistently detected in both unfertilized and fertilized eggs, XPA and XPC were never detected, while all other genes were detected in only one cell type.

This project represents an important step for understanding the molecular mechanisms linking paternal exposure to tobacco smoke, induction of genetic lesions in sperm, DNA repair capacity of the fertilized egg, and the risk of paternally transmitted chromosomal abnormalities. Work performed under the auspices of the U.S. DOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48.

H2

Fertility, smoking and early mammalian development

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We have previously reported that the structure of the oviduct and the abundance of the vasculature in corpora lutea of females are affected by inhalation of mainstream and sidestream cigarette smoke (Reprod Toxicol 9:513, 1995). Subsequent studies have shown that smoke solutions alter ciliary beat frequency and oocyte pickup rate of oviducts and also disrupt angiogenesis when assayed using chick chorioallantoic membranes (CAMs). Our current goal is to isolate and identify the chemicals in smoke solutions that produce these adverse effects on the oviduct and corpus luteum. Our strategy has been to partially purify and concentrate the chemicals in mainstream and sidestream smoke solutions using Bond Elut solid phase extraction cartridges, then identify the chemicals in the partially purified solutions using gas chromatography-mass spectroscopy (GC-MS). Once chemicals are identified, they will be tested using the oviductal and angiogenesis assays to determine which inhibit oocyte pick-up and blood vessel development. Both polar and non-polar solid phase extraction cartridges were first screened for their ability to retain components that inhibit both oocyte pick-up by the oviduct and angiogenesis in the CAM. After loading a cartridge with 1 ml of either mainstream or sidestream smoke solution, the cartridge was eluted with methanol. Examination of the eluents with HPLC revealed that the use of a single solid phase cartridge to partially purify the smoke solutions had significantly reduced the number of chemicals to be investigated. The eluent was gently blown down with nitrogen and the residue resuspended in culture medium, which was then tested either in the oviductal or angiogenesis assay. Based on data from this initial screen, four cartridges were identified that retained almost 100% of the toxicity in the oviductal and angiogenesis assays. Mainstream and sidestream smoke solutions that have been partially purified on these four solid phase extraction cartridges are currently being subjected to GC-MS to identify the chemicals in each solution. Many of the chemicals in the mainstream solutions have already been identified using MS, although several of the spectra will require further analysis. As chemicals are identified, they are being purchased in pure form from commercial vendors and tested in the oviductal and angiogenesis assays. We will then do dose response experiments with each toxicant to determine its effective dose in vitro and in vivo. Once these toxicants are known, we may be able to reduce their levels in smoke or provide medications to patients and thereby minimize the adverse affects of cigarette smoke toxicants on fertility. Our results will have broader applications as well since angiogenesis is required in embryonic and fetal development, adult female reproductive organs, wound healing, and certain diseases such as tumor growth, psoriasis, and retinopathy. Thus data obtained in this study may ultimatley lead to health improvements in many areas including reproduction, development, wound healing, and control of certain diseases, which all normally require extensive angiogenesis.

POSTER SESSIONS
Session H: Cardiovascular & Reproductive Health Effects

H3
Transport and disposition of nicotine in the human placenta

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Smoking during pregnancy is considered a major health risk for the fetus. It has been associated with low birth weight, perinatal death, alterations in fetal cardiorespiratory status, problems with long term growth, and sudden infant death syndrome. During pregnancy the placenta provides nourishment to the fetus and removes waste products from the fetal circulation. Moreover, it serves as a barrier against exposure to high levels of toxic agents which may include those present in tobacco smoke. Nicotine is the main pharmacologically active compound present in tobacco smoke. The goal of our studies is to characterize the role of specific proteins that may be involved in the metabolism (cytochrome P450s or CYPs) and transport (multidrug resistance proteins like P-glycoprotein or MRPs) of nicotine in the placenta, thus controlling fetal exposure to nicotine.

The cytochrome P450 enzymes are a large family of metabolizing enzymes that can break down many drugs and environmental toxins. We hypothesized that CYPs in the human placenta were involved in converting nicotine to a less toxic compound, cotinine, that could then be eliminated from the body. In the human liver, a single CYP enzyme, CYP2A6, has been found to be involved in this reaction; therefore, we presumed that CYP2A6 was also the main nicotine-metabolizing enzyme in the placenta. Moreover, we hypothesized that nicotine metabolism would be increased in placentas from smoking women compared to non-smoking women due to the increased levels of nicotine in the mother and the protective response of the placenta to protect the fetus from this chemical. Analyses of nicotine metabolism have shown that placenta failed to significantly metabolize nicotine to cotinine. Furthermore, CYP2A6 protein levels were found to be low and comparable in human placentas obtained from smokers and non-smokers.

A second mechanism that could control the exposure of the fetus to nicotine is the transport of this compound across the placenta. The multidrug resistance proteins, P-glycoprotein and MRPs, play important roles in the transport of numerous drugs into and out of cells. We proposed that they were also involved in "pumping" nicotine into and/or out of the placenta. We have analyzed protein levels of these transporters to ascertain if their expression is influenced by the smoking status of the mother. Our studies have found low but similar levels of P-glycoprotein in placentas of smoking and non-smoking women. Interestingly, protein levels of MRP1 were decreased in placentas from smokers relative to non-smokers. We have also used model cellular transport systems to determine whether nicotine interacts with these transporters. So far our data indicates that nicotine is not actively transported by P-glycoprotein or MRP2.

Another multidrug resistance transporter, mitoxantrone resistance transporter (MXR), is highly expressed in placenta and effluxes a variety of drugs out of cell lines that overexpress this transporter. We are currently analyzing whether this novel transporter interacts with nicotine and therefore could be implicated in regulating the access of nicotine to the fetus.

Our results suggest that CYP-mediated metabolism and transport by multidrug resistance proteins (P-glycoprotein and MRPs) do not influence nicotine disposition in the human placenta. It is essential to obtain a detailed characterization of how the placenta determines fetal exposure to nicotine in order to understand the consequences of fetal exposure to tobacco smoke.

H4
Environmental tobacco smoke and depletion of gonadal steroids exacerbates atherosclerosis in female LDL receptor-deficient mice

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Epidemiologic evidence suggests that postmenopausal women who smoke are protected from heart disease by hormone replacement therapy to a greater extent than nonsmokers. To determine if this relationship holds for women exposed to environmental tobacco smoke (ETS) and to define the interactions between ETS exposure and estrogen treatment, we chose to use mice susceptible to atherosclerosis. We had previously shown that removing the ovaries from young female low density lipoprotein receptor-deficient (LDLR-/-) mice resulted in a doubling of aortic atherosclerotic lesion and that estrogen replacement reversed the increase (Marsh *et al.*, J. Lipid Res. 40:893). For this study, aimed at examining postmenopausal conditions, mature female LDLR-/- mice were castrated, fed a high-fat, high-cholesterol diet (HFD) for 12 weeks and exposed for 10 of the 12 weeks to filtered air (FA) or to aged and diluted sidestream cigarette smoke (ADSS) as a surrogate for ETS. Sham operated females exposed to FA or ADSS served as controls. At termination of the study, aortae were collected for lesion analysis; uteri were collected for weight determination (a measure of the effectiveness of ovariectomy); and plasma samples were collected for lipid analyses.

In mice subjected to low-dose ADSS (1 mg/m^3 total suspended particulates), there was an increase in aortic lesion area compared with FA controls only in the ovariectomized (OVX) females; however, a statistically significant increase in atherosclerosis was seen in both OVX and intact females exposed to high-dose ADSS (5 mg/m^3 total suspended particulates). An ADSS-mediated decrease in uterine weights in the intact females prompted us to measure circulating estrogen levels. The estrogen levels for 14 of 15 high-dose ADSS-exposed intact females were in the lowest quintile, suggesting that exposure to tobacco smoke decreases circulating estrogen levels in female mice as does smoking in women. This decrease in estrogen levels may contribute to the atherogenic effects of ADSS. In conclusion, in mature female LDLR-/- mice, we found a dose-dependent, ADSS-mediated increase in atherosclerosis that was exacerbated by the absence of circulating ovarian steroids. This supports our hypothesis that estrogen replacement will confer protection from cardiovascular disease to women exposed to "second hand" smoke. Studies are currently underway to examine the contributions of estrogens and progestins to the atherogeneic profiles in the ADSS-exposed mice.

H5

A comparison of total plasma antioxidant status of passive smokers, smokers and nonsmokers - with adjustment for dietary intake of antioxidants

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Background: Cigarette smoke contains large quantities of reactive free radicals, which cause oxidative damage to lipids, proteins, and DNA. It has been hypothesized that oxidative damage to these macromolecules is a risk factor in the development of cardiovascular diseases and cancer. In vitro studies have shown that free radicals in cigarette smoke decrease certain plasma antioxidants. Thus, low levels of antioxidants may be a risk factor for cigarette smoke related diseases in smokers and in passive smokers.

Objective: In this study we compare plasma antioxidant levels of passive smokers and active smokers with nonsmokers to investigate whether not only active smoking but also exposure to cigarette smoke decreases plasma antioxidants.

Design: Ninety smokers, 42 passive smokers and 40 nonsmokers provided fasting plasma samples. The samples were analyzed for ascorbic acid, α-tocopherol, γ-tocopherol, α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, total carotenoids and retinol. Subject groups were compared by one-way ANOVA with adjustment for race, sex, age, bmi, alcohol intake, and dietary antioxidants. For lipophilic antioxidants we additionally adjusted for triglycerides.

Results: Smokers and passive smokers have statistically significantly lower levels of plasma β-carotene than nonsmokers (p=0.007, p=0.002 respectively). Smokers have significantly lower mean plasma levels of ascorbic acid than passive smokers and nonsmokers (p=0.007, p=0.007). Middle aged smokers (35-54 years of age) have significantly lower levels of β-cryptoxanthin and lutein/zeaxanthin than passive smokers and nonsmokers. Since we controlled for dietary intake of antioxidants, the results do not reflect differences in dietary habits of the subject groups. Data on all the other micronutrients will be reported at the meeting.

Conclusions: These results show that passive smokers as well as active smokers have a higher turnover of certain antioxidants. Therefore, risk of cigarette smoke related diseases might be reduced with an adequate intake of antioxidants either through a diet high in antioxidants or through antioxidant supplements.

H6

Covalent modification of platelet cell surfaces

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Platelets, the blood cells responsible for blood coagulation, are critical in healing and in the prevention of blood loss. However, they may play an ominous role involving cardiovascular disease (CVD), the leading cause of death in the United States. 4(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a procarcinogen present in tobacco and requires metabolic activation to form DNA adducts. Our question is whether unactivated NNK can covalently modify any of the large variety of proteins preseon platelet cell surfaces. Radiolabeled proteins, if formed from ^{14}C-NNK, would signal the presence of tobacco-induced cell surface antigens, thus providing evidence for a relationship between smoking and CVD.

^{14}C-NNK labeled proteins can be detected by two methods, both involving polyacrylamide gel electrophoresis to separate proteins by mass. One method uses a soluble gel matrix to quantify ^{14}C-NNK by liquid scintillation. However, when labeling intact cells versus isolated membrane proteins, we find approximately 8 orders of magnitude less binding of ^{14}C-NNK per cell. To detect such low levels of ^{14}C-NNK binding intact cells, the other method for ^{14}C quantification, analysis by Accelerator Mass Spectrometry (AMS) must be used. We have found by using both methods that ^{14}C-NNK binds a small platelet membrane protein (or a subunit of a much larger protein) with an apparent molecular weight of 45-50 kDa.

POSTER SESSIONS
Session H: Cardiovascular & Reproductive Health Effects

H7
Inhibition of nitric oxide synthesis by cigarette smoke

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Cigarette smoking is associated with an increased risk of cardiovascular disease that may involve increased hypertension and platelet aggregation, both of which may be caused by decreased endogenous nitric oxide (NO) formation. Previous studies have shown that cigarette smoking reduces endogenous NO formation in smooth muscle tissues and that levels of exhaled NO are reduced in smokers. NO is produced by nitric oxide synthase (NOS) utilizing L-arginine as a substrate. In vitro studies have shown that NOS activity in tissues and cells is decreased in the presence of cigarette smoke extracts (CSE). The exact pathophysiological mechanisms underlying these effects are, however, incompletely understood. One potential explanation for reduced NOS activity by exposure to CSE may be the formation of adducts of the NOS substrate L-arginine. This may decrease available L-arginine levels for NO synthesis or, result in formation of adducts that competitively inhibit NOS. Recent observations that endothelial dysfunction by cigarette smoke can be prevented by dietary L-arginine supplementation seem consistent with this hypothesis. We investigated the direct reaction between CSE and L-arginine by exposing L-arginine solutions to filtered CSE and monitoring L-arginine depletion and the formation of adducts. Analysis by HPLC and mass spectroscopy indicated that the major adduct is formed by the direct reaction between L-arginine and acetaldehyde, a major cigarette smoke component. In addition, HPLC analysis of a solution containing L-arginine and acetaldehyde revealed a product with an identical retention time. We prepared purified acetaldehyde-L-arginine adduct by G-15 column chromatography, and investigated its ability to inhibit NOS activity. The adduct was shown to competitively inhibit purified NOS incubated with submaximal levels of L-arginine. Identification of L-arginine adducts may serve as biomarkers of smoking-related cardiovascular dysfunction, and would give additional support for L-arginine replacement strategies.

H8
Carbon monoxide as a modulator of myocardial function

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Despite the awareness of carbon monoxide (CO) as a deadly poison in environmental tobacco smoke (ETS) and pollutant, the precise cellular damage remains an open question. In excess, CO will readily displace O_2 in blood, and the cells will just suffocate. Although the CO level in ETS is low, the reduced oxygen delivery will still compromise cardiac function. This common view overlooks the interaction of CO within the cell. Even at very low concentration, CO can bind tightly to the intracellular oxygen storage protein, myoglobin (Mb) and might reduce the cell's ability to maintain a properly energized state. Such interaction with Mb could significantly affect heart function and may be a significant basis for the observed risk of heart disease in subjects exposed to ETS. The study focuses on how Mb inhibition with CO impacts on the cellular response in normal, ischemic, and postischemic hearts.

In perfused rat hearts that received 10% CO, sufficient to inhibit 86% of the cellular Mb, the myocardial oxygen consumption, heart rate, and developed pressure exhibit no significant alteration. However, lactate levels increased above the level observed when the hearts receive the same amount of N_2. The increase in lactate suggests that Mb inhibition indeed produces a functional hypoxia or alteration in cellular bionergetics independent of the available oxygen supply. Moreover, even though the presence of CO does not alter the onset of ischemia nor reduce the steady state recovery level of cardiac function or metabolism, it decreases the time to normal sinus rhythm in the postischemic heart.

These observations confirm the hypothesis that CO can directly interfere with cellular function, even at a low level of exposure in ETS and raise a provocative question about the threshold level for acute vs. chronic CO toxicity. Further studies will distinguish the CO inhibition of Mb vs. a direct CO interaction as the basis for the observed cellular changes and the risk of cardiovascular disease.

H9

Environmental tobacco smoke exposure increases permeability and glycoxidative damage to the artery wall

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Environmental tobacco smoke (ETS) has been strongly linked with the development of atherosclerosis. Our work has shown that ETS modifies low-density lipoproteins (LDL) and the artery wall such that there is increased LDL uptake in the artery wall, a progenitor of atheroma formation. These studies also suggested that the endothelial layer of arteries was injured by ETS and that permeability was increased. Other studies have suggested that tobacco smoking increases tissue glycoxidative damage (the synergistic interactions of glycation and oxidation). The goal of this project was to determine the actions of ETS on the artery wall including endothelial layer permeability and glycoxidative damage and vascular protective effects afforded by the antioxidant vitamin E.

Intact female rats were exposed to ETS (nicotine 5.3 mg/m^3, carbon monoxide 90 ppm, total suspended particulates 30.8 mg/m^3) or filtered air for 6 weeks (6 hours/day, 5 days/week) in the exposure chambers at UC Davis. The animals were fed either a high vitamin E diet (300 IU/kg) or standard vitamin E diet (30 IU/kg). At the end of the 6 weeks of exposure, ETS-treated rats weighed significantly less than filtered air-treated rats (251 ± 9 vs 304 ± 15 gm). The rate of accumulation of fluorescently labeled dextran in the carotid artery wall was determined by quantitative fluorescence microscopy. Exposure of the animals to ETS increased dextran permeability 70% as compared to exposure to filtered air (2.61 ± 0.28 vs 1.83 ± 0.11 ng/min/cm^2). The presence of increased vitamin E in the diet did not prevent increased endothelial layer permeability in animals exposed to ETS. Aortas from these animals were examined for pentosidine, a highly specific marker of glycoxidative damage. Pentosidine was increased about 3-fold in the ETS-exposed animals (9.71 ± 1.39 vs 3.55 ± 0.66 ng/mg hydroxyproline).

In sum, our studies have shown that ETS has multiple actions on the artery wall. In addition to its actions on LDL, exposure to ETS could injure artery endothelium and serve as an initiating event in atheroma formation in individuals exposed to environmental tobacco smoke. Further studies are needed to identify the actions of glycoxidative damage induced by exposure to ETS on the artery wall.

H10

Nicotine stimulates atherogenesis by enhancing plaque vascularization

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Tobacco exposure is a well-known risk factor for the development and progression of atherosclerosis, a process that requires angiogenesis for sustained growth of the lesion. Surprisingly, we recently have shown that nicotine is a potent angiogenic agent, an effect that is mediated in part by cyclooxygenase. Accordingly, we investigated whether the enhanced atherosclerosis in smokers might in part be due to nicotine's ability to promote intimal vessel growth.

Methods: ApoE-deficient mice were fed a 0.15% cholesterol diet. At 20 weeks, mice were divided into 4 groups and treated for 20 weeks with, nicotine (0.1 g/L drinking water) nicotine + rofecoxib (COX2 inhibitor with anti-angiogenic properties), rofecoxib only, or placebo. Plaque formation in the aortic origin was investigated for plaque growth and capillary density.

Results: At 40 weeks, median LDL cholesterol and body weight were similar for the four groups whereas mortality in the nicotine-treated group was significantly higher (P<0.012). Mean plaque area at 20 weeks prior to randomization for treatment was 0.18 mm^2 (95% CI 0.12-0.29). At 40-week follow-up, median plaque area in the control group had increased to 0.48 (95% C1 0.29 - 0.63) mm^2. In the nicotine group, the increase in lesion growth was twice as great with a median plaque area of 0.81 mm^2 (95% CI 0.50-0.84; P<0.001). The acceleration of lesion growth in nicotine-treated animals was abrogated by rofecoxib (a COX-2 inhibitor and antagonist of angiogenesis); in these animals, plaque area was 0.44 mm^2 (95% CI 0.22-0.52) and did not significantly differ from control group (P=0.61). Animals treated with rofecoxib alone manifested a median plaque area of 0.39 mm^2 (95% C1 0.25-0.53) that tended to be less than control animals (P=0.15).

Aortic sinus plaques isolated from control and treated animals were also examined for the presence of intimal vessels. The acceleration of plaque growth in nicotine-treated animals was associated with an increase in plaque vascularization. The percentage of plaques that contained at least one intimal vessel was significantly higher in nicotine-treated mice (37% versus 16%; nicotine versus control; P<0.001). This increased plaque vascularization in the nicotine group was reduced to the level of the control group when rofecoxib was co-administered with nicotine (21%; P=0.72 versus control). Animals treated with rofecoxib alone showed a significantly lower plaque vascularization compared to control (8% versus 16%; rofecoxib versus control; P=0.015). The plaque growth observed was not related to increased smooth muscle cell migration and proliferation in aortic lesions. The median smooth muscle cell contents of lesions from control mice were similar (26.6% vs. 25.2%; P=0.68).

Conclusions: Nicotine significantly enhanced atherogenesis in ApoE-deficient mice. The COX2 inhibitor rofecoxib abrogated the effect of nicotine on plaque growth, consistent with out previous observation that cyclooxygenase mediates the angiogenic effect of nicotine. The effect of nicotine to accelerate atherosclerosis may be in art due to its angiogenic action.

POSTER SESSIONS
Session H: Cardiovascular & Reproductive Health Effects

H11
Effect of smoking on left ventricular remodeling following myocardial infarction

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Background: Anecdotal evidence suggests that smokers experience impaired wound healing. Recent studies have shown that rats exposed to nicotine at the time of MI have worsened left ventricular (LV) remodeling. Therefore, we studied the effect of smoking on LV remodeling & recovery of function in patients following myocardial infarction (MI).

Methods: We analyzed 68 serial echocardiograms (1088 LV wall segments) from 17 consecutive patients with a first anterior wall (AW) MI who underwent angiography (11 nonsmokers, 6 smokers; age 70±11 vs 59±8 yrs, p=0.05). Patients had echocardiograms within 48 hrs of MI & again at 1, 3, & 6 mos post-MI. Echocardiograms included measurement of LV dimensions, ejection fraction, cardiac output, systolic & diastolic LV volumes, LV mass, # of infarct-related LV segments, mean infarct-related wall motion score, & mean global LV wall motion score (1=nl, 2=mild-mod hypo, 3=severe hypo, 4=akinesis). Catheterization results were also analyzed.

Results: There were no significant differences between nonsmokers & smokers in baseline LV end-diastolic volume (122 vs 118 cc), cardiac output (5.9 vs 5.0 l/m), EF (49 vs 44%), & LV mass (280 vs 300 g, p=ns for all). The number of infarct-related LV segments was slightly lower in nonsmokers (7.2 vs 8.8) but it was not significantly different (p=0.24). Baseline infarct-related wall motion (IRWM) scores were similar in both groups (2.6 vs 2.5, p=ns), as were the global LV wall motion (WM) scores (1.7 vs 1.8, p=ns). Over a 6 month period, however, nonsmokers showed a significant improvement in the IRWM score (2.6 to 1.9, p=0.03) while the smoking group showed no change (2.5 to 2.6, p=ns). The non-smokers' global LVWM score improved from 1.7 to 1.4, p=0.06), while the smokers' global score was unchanged (1.8 to 1.9, p=ns).

Conclusions: These preliminary results suggest that although nonsmokers & smokers present with anterior wall myocardial infarctions of similar initial size, smokers show significantly less recovery of function in the infarct-related territory over a period of 6 months. In addition, smokers do not exhibit the trend toward improvement in global LV systolic function that is seen in nonsmokers following anterior wall myocardial infarction.

H12
Novel strategy for protecting neurons from injury in stroke

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One of the main adverse consequences of tobacco use is increased risk of hypoxic-ischemic brain disease, including the global ischemia of cardiac arrest, and the focal ischemia of a stroke. There is now sufficient knowledge concerning the biology of the resulting neuron death to design protective interventions. The focus of my laboratory has been to design gene therapy strategies to block neuron death post-insult. The approach involves use of viruses (such as herpes simplex) which preferentially infect neurons. Viral genes that lead to dangerous replication are removed and replaced with potentially neuroprotective genes. This "vector" is then introduced into the brain.

We have explored the protective potential of some 20 genes against various neurological insults, both as modeled in neuronal cell cultures, and in animals. The present work concerns one novel intervention. Following ischemic injury, a subset of neurons that die do so by "apoptosis," or programmed cell death. Such cellular suicide normally occurs during development, or when the immune system targets an infected cell, and the same pathway is maladaptively triggered in some injured neurons. "Apoptosis-inhibitor" genes exist in mammalian cells, and we have shown that the delivery of one of these (called Bcl-2) can decrease damage.

A main defense of the body against viral infection is to trigger apoptosis in infected cells (killing them before the virus can replicate). As a countermeasure, viruses have evolved an array of anti-apoptotic genes. As one facet of the TRDRP grant, we are exploring the neuroprotective potential of these viral genes. The rationale has been two-fold, beyond merely exploring another potential route of neuroprotection. First, it is given wisdom in virology that these are powerfully effective genes. Second, insofar as we have been using a viral vector system to deliver a *mammalian* anti-apoptotic gene, there may be unrecognized benefits to using a viral system to deliver *viral* anti-apoptotic genes, in terms of increased efficiency or potency.

We initially focused on one called Ksbcl-2; this is found in a herpes virus implicated in causing Kaposi's sarcoma, and is structurally related to the mammalian bcl-2 gene in a way suggesting that it should be even more neuroprotective. We have also expanded our work to include three other viral anti-apoptotic genes ("p35" from baculovirus; "CrmA" from cowpox virus; "gamma 34.5" from herpes simplex virus 1). We have observed: a) Ksbcl-2 decreases the neuron death in a culture model of anoxia. Despite this, it is less protective than anticipated, being no more so than its mammalian counterpart, bcl-2. We are now investigating why this is the case. b) The other genes are protective against culture models of anoxia and excitotoxicity, as well as against models of neuron death in the whole rat. c) Surprisingly, after careful study, we find no evidence that such protection actually involves blocking apoptotic death; instead, these genes block other cell death pathways. d) Preliminary data suggest that, instead, they protect by stabilizing neuronal energetics during insults. Our ongoing studies explore the protective potential and mechanisms of action of these genes.

H13

Generation and atherosclerosis studies of transgenic mice with macrophage-specific urokinase overexpression

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Smoking is a risk factor for the development of atherosclerosis and its complications, including coronary artery disease, acute myocardial infarction, and aortic aneurysms. It has been hypothesized that excessive protein-degrading or "proteolytic" activity in the artery wall may contribute to the development of atherosclerosis and its complications. A substantial body of work has associated high levels of plasminogen activators (a type of proteolytic enzyme) with human atherosclerosis. However, it is not known whether plasminogen activators cause atherosclerosis or whether they are part of the body's defense system against atherosclerosis. It is important to understand the association between plasminogen activators and atherosclerosis because plasminogen activators are used as therapeutic agents in humans. Indeed, our group has proposed using gene therapy with plasminogen activators as a means of treating and preventing atherosclerosis. As part of our efforts to test the feasibility of using gene therapy with plasminogen activators to treat and prevent atherosclerosis, we generated transgenic mice that expressed high levels of urokinase plasminogen activator (uPA) in their macrophages. Macrophages are cells that are abundant in atherosclerotic arteries, so mice with high levels of urokinase expression in their macrophages should also have high levels of urokinase expression in their atherosclerotic arteries. We are using these transgenic mice to test whether increasing the level of urokinase in atherosclerotic arteries prevents or worsens atherosclerosis.

We began by generating three lines of transgenic mice that express varying levels of urokinase in their macrophages. We first confirmed that the mice had elevated urokinase expression primarily in macrophages rather than in other cell types. We then bred the urokinase transgene into the genetic background of apolipoprotein E deficiency. Apolipoprotein E -deficient mice develop spontaneous atherosclerosis, which permits us to determine the effect of urokinase expression on atherosclerosis development. Apolipoprotein E -deficient mice can also develop complications of atherosclerosis, including myocardial infarction and aortic aneurysms. This predisposition allows us to determine whether urokinase can prevent or trigger these complications. We are carrying out a series of studies in the three lines of mice in which we are determining whether increased urokinase expression affects the development of atherosclerosis and the occurrence of its complications. We will present the available data, which so far appear to suggest that urokinase can trigger the complications of atherosclerosis.

We will complete this study during the next few months. We anticipate that our results will reveal an important role for urokinase in the natural history of atherosclerosis. While these results may lessen enthusiasm for using urokinase for gene therapy for atherosclerosis, they may provide a better understanding of the mechanisms through which atherosclerosis develops. We anticipate that this understanding will be applied in order to develop better treatments and preventive strategies for atherosclerosis. These strategies should be of benefit to tobacco users, who are particularly likely both to develop atherosclerosis and to suffer from its complications.

I1

Nicotinic modulation of dopamine release during development

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Nicotine is the major psychoactive component in tobacco that maintains smoking. Nicotinic modulation of central dopaminergic (DAergic) transmission, in particular the mesolimbic pathway that originates from the ventral tegmental area (VTA) and innervates the ventral striatum, is responsible for the reinforcing properties of this drug. Teenagers seem to be especially susceptible to the addictive properties of nicotine, which raises the question of whether adolescents are more sensitive to the addictive properties of nicotine. In the present study, we have used both anatomical and biochemical functional assays to determine whether nAChRs present within the DAergic neurons of the developing rat substantia nigra compacta (SNc) and VTA are different than the adult receptors. Using [^{35}S]-labeled riboprobes, we have constructed a preliminary developmental profile for nAChR subunits α3-α7 and β2-β3. α3, α4, α5 and β2 displayed a biphasic developmental profile, with a peak of expression prenatally, followed by another peak of expression at an early postnatal age. There was a distinct peak of expression at postnatal day 21 for α5, α6 and α7 mRNAs. The profile for β3 mRNA differed from the other subunits in that it did not show any peaks of expression in any developmental time point. There was not a significant gender difference in the developmental profile of these subunits. Analysis of [^{3}H]nicotine binding within the SNc and VTA closely followed the developmental expression of the α4 and β2 subunit mRNAs. Nicotine stimulated [^{3}H]DA release from striatal slices was performed to functionally characterize nAChRs present on the DAergic nerve terminals of developing brain. In males, there was a gradual increase in nicotine stimulated maximal release of [^{3}H]DA from the developing striatum, with adult values reached at postnatal day (P) 21. In conjunction with an increase in maximal release, there was an increased sensitivity to nicotine with age, although this was only seen in the dorsal striatum. The ventral striatum of P30 and P40 rats show an increased sensitivity to nicotine compared to the adult. The present preliminary data suggests that nicotine may be more effective in stimulating [^{3}H]DA release from the ventral striatum of adolescent animals.

In the future work, we will focus on examining gender differences in the nicotine stimulated [^{3}H]DA release profile. In addition, as the animal develops, the release displays a biphasic profile, with the second, less potent component believed to be mediated by glutamate. We will determine differences in the developmental profile of the two phases, as this would provide an additional mechanism by which nicotine may differentially regulate the adolescent reward pathway, thus affording more opportunities for the development of effective therapeutic agents aimed towards facilitating the quitting process which many adolescents find difficult to do.

I2

Chronic nicotine exposure during a developmental "critical period" disrupts synaptic development in rat auditory neocortex

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The goal of our work is to determine how nicotinic acetylcholine receptors (nAChRs) contribute to normal development of auditory cortex, and how exposure to nicotine might affect this development. Prenatal nicotine exposure in humans subsequently produces auditory and auditory-related cognitive deficits in children. A developmental period that may be critical to this effect follows the onset of hearing in the third trimester (and in the second postnatal week for rats), and involves the growth of auditory nerve fibers into the auditory cortex and the subsequent formation of cortical neural circuitry that mediates auditory perception throughout life. Importantly, this period is also characterized by a dramatic increase in anatomical markers for the neurotransmitter acetylcholine (i.e., markers for acetylcholinesterase and alpha-7 nAChRs) in auditory cortex.

Recently, we demonstrated that alpha-7 nAChRs selectively enhance excitatory postsynaptic potentials (EPSPs) mediated by *N*-methyl-D-aspartate receptors (NMDARs) in rat auditory cortex (Aramakis and Metherate, 1998). This effect is most prominent during the second postnatal week and does not occur after the third week. To investigate the developmental role of this regulation and to determine the effects of exogenous nicotine on cortical development, in the present study we determined if manipulating nAChR function at specific times during the first four weeks after birth could alter subsequent neural function. Rat pups were injected twice daily with 1 or 2 mg/kg nicotine (0.35 or 0.7 mg/kg free base) or saline during the first, second, or fourth postnatal weeks (i.e., before, during, or after the peak upregulation of nAChRs). Glutamate EPSPs were measured during intracellular (whole-cell) recordings from neurons in layers III-IV of brain slices prepared at least 15 hours after the last injection. Chronic nicotine exposure (CNE) during weeks 1 or 4 did not affect synaptic function. However, CNE during week 2 resulted in EPSPs with unusually long durations, multiple peaks, and twofold enlarged NMDAR components. These changes remained significant even 10 days after CNE. Rapid application of nicotine to neurons during week 2, which normally enhances NMDAR EPSPs, produced only weak effects after CNE. Receptor binding studies showed that CNE-induced EPSP alterations occurred in the absence of altered alpha-7 nAChR numbers or agonist binding affinity. Thus, altered stimulation of nAChRs by CNE during week 2 disrupts the development of glutamate synapses in rat auditory cortex.

This study indicates that early exposure to nicotine can disrupt neural development in auditory cortex. Such an effect, if it occurs in humans, may underlie the diminished auditory ability of infants exposed to nicotine and the subsequent deficits on auditory-related cognitive tests in older children. Future studies must determine how the disruption of EPSP development affects subsequent auditory function in rats, and whether a similar effect occurs in humans exposed to nicotine from tobacco.

POSTER SESSIONS
Session I: Nicotine Dependence

I3
Pretreatment with clozapine prevents nicotine withdrawal

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Smoking cessation in nicotine-dependent humans results in an abstinence syndrome, which includes affective and somatic signs of withdrawal that contribute to the high relapse rate in smoking cessation efforts. Nicotine withdrawal is characterized by a symptom (i.e., anhedonia or "diminished interest or pleasure" in rewarding stimuli) that is also one of the negative symptoms of schizophrenia. Clozapine is more effective in the treatment of the negative symptoms of schizophrenia than neuroleptics. Based on the phenomenological similarity between symptoms of nicotine withdrawal and the negative symptoms of schizophrenia, it is hypothesized here that clozapine treatment may be effective against nicotine withdrawal symptoms.

The intracranial self-stimulation paradigm was used to assess affective changes associated with nicotine withdrawal. Elevations in brain reward thresholds are interpreted as a decrease in the reward value of the stimulation and are considered a measure of the symptom of "anhedonia". Observational methods were used to assess somatic signs associated with nicotine withdrawal. In Experiment 1 the effects of clozapine administered during nicotine withdrawal were studied. Subjects' were prepared with osmotic minipumps (9 mg/kg/day nicotine hydrogen tartrate salt or saline). On day 7 the minipumps were removed and the rats' brain reward thresholds and somatic signs were assessed daily after either clozapine (0.75-3 mg/kg) or vehicle treatment for six days. Nicotine withdrawal resulted in elevated brain reward thresholds and increased number of somatic signs compared to saline-exposed rats. Clozapine at the dose of 3 mg/kg induced threshold elevations and increased the number of somatic signs in both nicotine and saline withdrawing subjects. Administration of a low dose of clozapine (1.5 mg/kg) did not affect the magnitude and duration of nicotine withdrawal. Most interestingly, the lowest dose of clozapine (0.75 mg/kg) reversed the increased number of nicotine withdrawal somatic signs without having an effect on the threshold elevations. In experiment 2 the effects of 10 days of chronic clozapine pretreatment (3 mg/kg; twice a day) before exposure to nicotine were studied. Brain reward thresholds and somatic signs were assessed between the morning and evening clozapine injections. Clozapine did not induce changes in brain reward thresholds, while the total number of somatic signs was increased during the 10 days of clozapine pretreatment. Somatic signs returned to baseline levels the day after the last clozapine injection. Then, subjects were prepared with nicotine or saline containing osmotic minipumps that were removed on day 7. Clozapine pretreatment prevented both the threshold elevations and the increases in somatic signs associated with nicotine withdrawal. In saline treated rats clozapine did not induce any changes in either in brain reward thresholds or somatic signs.

The findings that clozapine prevented nicotine withdrawal is analogous to clozapine preventing relapse to a psychotic episode in schizophrenia patients. Thus, the present experimental outcome suggests that nicotine withdrawal may be an animal model of the negative symptoms of schizophrenia. Finally, the results suggest that clozapine treatment may increase success in smoking cessation efforts of schizophrenia patients by alleviating nicotine withdrawal symptoms and decreasing the probability of relapse to smoking among these patients.

I4
Role of α7 nicotinic receptors in nicotine self-administration and nicotine withdrawal in the rat

Paterson, Neil / Markou, Athina
The Scripps Research Institute

Nicotine has acute positive reinforcing effects (i.e. pleasurable short-term effects), such as euphoria and improved cognitive function. Intravenous self-administration of nicotine has been shown to be stable and replicable in a wide variety of animals, including rats, dogs, and non-human primates. This behavior reflects the acute positive reinforcing effects of nicotine. In addition, there is a well defined withdrawal syndrome in humans and animals after cessation of chronic nicotine exposure. This withdrawal syndrome has an affective (emotional) component and a somatic component. In the rat, the somatic component includes behaviors such as gasps, yawns, blinks, and abdominal writhes ("somatic signs"). Brain reward function, as measured by intracranial self-stimulation, provides an index of the emotional component of nicotine withdrawal. It is hypothesized that both the acute effects of nicotine, and the symptoms associated with withdrawal from chronic exposure to nicotine contribute to the addictive properties of nicotine. Several types of nicotinic receptor have been identified, although the functions of specific receptor subtypes remain unclear. The purpose of the present study was to investigate the role of α7 nicotinic receptors in nicotine self-administration and nicotine withdrawal by using the selective α7 nicotinic receptor antagonist methyllycaconitine (MLA). The effects of MLA on nicotine self-administration were tested. It has been shown previously that nicotine self-administration is decreased by the administration of non-selective nicotinic receptor antagonists. The effects of MLA on brain reward function and somatic signs in chronically nicotine-exposed rats were also assessed. It has been previously shown that brain reward function is potentiated by acute nicotine administration, whereas withdrawal from chronic nicotine administration and administration of non-specific nicotinic receptor antagonists in chronically nicotine-exposed rats is associated with impaired brain reward function. Further, administration of non-specific nicotinic receptor antagonists precipitated a significant increase in the number of somatic signs of withdrawal in chronically nicotine-treated rats.

The administration of the two highest doses of MLA significantly reduced intravenous self-administration at two different doses of self-administered nicotine. Nevertheless, MLA had no effect on brain reward function or the number of somatic signs in rats chronically exposed to either nicotine or saline. These results suggest a potential role for the α7 receptor in the acute positive reinforcing effects of nicotine, although there is some doubt regarding the specificity of MLA for the α7 nicotinic receptor at the effective doses. In addition, the results indicate no role for α7 nicotinic receptors in nicotine dependence as reflected in the lack of precipitation of the nicotine withdrawal syndrome, either in its affective (brain reward function) nor in its somatic components. Future work would include studying the effects of other nicotinic receptor agonists and antagonists to elucidate the function of the different nicotinic receptor subtypes.

This work is relevant to the development of drugs to treat nicotine addiction. Nicotine addiction, commonly manifested as cigarette smoking, is a major cause of morbidity and mortality in the world. The delineation of nicotinic receptor function will facilitate the development of drugs effective in treating nicotine addiction, and thus will significantly reduce both the human and economic costs of tobacco-related morbidity and mortality.

I5

Influence of duration of nicotine exposure and withdrawal history on severity and duration of nicotine withdrawal

Skjei, Karen / Markou, Athina
The Scripps Research Institute

The negative affective aspects of nicotine withdrawal are thought to contribute to the addictive properties of chronic nicotine. One way to assess the affective aspects of nicotine withdrawal is through the use of the brain stimulation reward paradigm that provides thresholds as a measure of reward. It has been shown previously that nicotine withdrawal results, not only in somatic signs of withdrawal, but also in elevations in brain reward thresholds that reflect diminished interest and pleasure in the electrical stimuli

The purpose of this study was to investigate the influence of previous withdrawal episodes and duration of nicotine exposure on the severity and duration of the negative affective aspects of nicotine withdrawal as assessed by intracranial self-stimulation reward thresholds. Somatic signs were also assessed at selected time points during the two experiments. Rats were prepared with bipolar stimulating electrodes in the lateral hypothalamus and trained in a discrete-trial current threshold procedure. In the first experiment, subjects underwent four successive periods of alternating nicotine exposure (via 7-day osmotic pumps) and spontaneous withdrawal (following pump removal). Nicotine-treated animals showed significant elevations in brain reward thresholds during all four withdrawal periods compared to controls. Furthermore, in nicotine-treated animals, the third and fourth withdrawals were associated with significantly longer lasting elevations in brain reward thresholds compared to the first and second withdrawals. A non-statistically significant trend of progressively augmented elevations in brain reward thresholds was also observed with successive withdrawals from nicotine. Nicotine-treated animals showed significantly more somatic signs than controls after the removal of the pumps, but no progressive augmentation was observed with successive withdrawals. In the second experiment, subjects prepared with 28-day nicotine- or saline-containing pumps were administered two 5-day series of daily injections of the nicotinic receptor antagonist dihydro-b-erythroidine hydrobromide (DHbE) or saline. Whereas DHbE injections had no effect in saline-treated rats, nicotine-treated rats that received DHbE showed significant elevations in brain reward thresholds compared to those that received saline injections. However, successive antagonist injections did not produce a progressive augmentation of the threshold elevation effect. In this second experiment, pumps were removed after four weeks of chronic nicotine or saline exposure and both brain reward thresholds and somatic signs were assessed. The duration of brain reward threshold elevations observed during spontaneous withdrawal in the nicotine-treated animals was longer than that seen following shorter nicotine exposures (i.e., 7 days; Experiment 1). Interestingly, both nicotine- and saline-exposed subjects that had received antagonist injections earlier in the experiment showed significantly fewer somatic signs after pump removal.

The results suggest that spontaneous nicotine withdrawal, as reflected in brain reward threshold elevations, becomes progressively longer with repeated withdrawal episodes. In addition, the duration of the negative affective aspects of withdrawal may be proportional to the duration of exposure to nicotine. Follow-up studies are currently being designed to further explore this effect by investigating the duration of withdrawal after various durations of nicotine exposure. A broader understanding of the factors that influence the severity and duration of the negative affective aspects of nicotine withdrawal may lead to more effective treatment strategies to facilitate nicotine abstinence in humans.

I6

Point mutant mice with hypersensitive a4 nicotinic receptors show dopaminergic deficits and increased anxiety

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Knock-in mice were generated harboring a leucine to serine mutation in the a4 nicotinic receptor near the gate in the channel pore. Mice with intact expression of this hypersensitive receptor display dominant neonatal lethality. These mice have a severe deficit of midbrain dopaminergic neurons, possibly because the hypersensitive receptors are continuously activated by normal extracellular choline concentrations. A strain that retains the neo selection cassette in an intron has reduced expression of the hypersensitive receptor and is viable and fertile. The viable mice display increased anxiety, poor motor learning, excessive ambulation that is eliminated by very low levels of nicotine, and a reduction of nigrostriatal dopaminergic function upon aging. These knock-in mice provide useful insights into the pathophysiology of sustained nicotinic receptor activation and may provide a model for Parkinson's disease.

POSTER SESSIONS
Session I: Nicotine Dependence

I7
Differential alterations in non-a7 and a7 nicotinic receptors in monkey striatum after MPTP treatment

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The Parkinson's Institute

Parkinson's disease (PD) is characterized by progressive degeneration of the nigrostriatal dopaminergic system leading to movement dysfunction. Evidence that nicotinic receptors may provide a therapeutic target for treatment includes the demonstration of an inverse relationship between smoking and the incidence of PD and studies that show that nicotinic agonists appear to alleviate parkinsonian symptoms.

Previous studies from this laboratory have shown selective changes in expression of nicotinic receptor subunit mRNAs in squirrel monkey (*Saimiri sciureus*) substantia nigra after administration of the dopaminergic toxin MPTP. In the present study, we used the nicotinic receptor ligands ^{125}I-epibatidine and ^{125}I-α-bungarotoxin to investigate changes in nicotinic receptor binding in the caudate-putamen of monkeys rendered parkinsonian with MPTP. Control animals exhibit a gradient in ^{125}I-epibatidine and dopamine transporter binding, increasing from dorsal to ventral putamen and from lateral to medial caudate. MPTP injection resulted in severe depletion of dopamine transporter binding (> 90%), but only a partial decline in ^{125}I-epibatidine binding to nicotinic receptors (39-69%). In addition, MPTP lesioning eliminated the dorsal-ventral and medial-lateral gradients seen in the nicotinic receptors, but not the dopamine transporter. ^{125}I-α-bungarotoxin binding, which labels α7-containing nicotinic receptors, surprisingly increased 60-136% in MPTP lesioned animals compared to control. These results indicate that there are differential alterations in expression of α7 and non-α7-containing nicotinic receptors after nigrostriatal degeneration.

The MPTP-lesioned nigrostriatal degeneration model for non-human primates causes changes in nicotinic receptors that are similar to those seen in Parkinson's disease patients. This work is relevant to treatment of Parkinson's disease with nicotinic receptor ligands, in particular since smokers exhibit elevated levels of nicotinic receptors and are less likely to develop the disorder. Additional experiments will be conducted to investigate the association of specific subtypes of nicotinic receptors with dopaminergic neurons. In addition, we will study the effect nicotine administration has upon receptor expression and protection against MPTP-induced parkinsonism.

I8
Nicotine induced neuroprotection against MPP^+ toxicity is mediated through a non-a7 receptor

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Epidemiological studies show that tobacco use is associated with a decreased incidence of Parkinson's disease, a neurodegenerative disorder associated with a loss of dopamine neurons in the substantia nigra. The agent in tobacco products responsible for the neuroprotective effect is not known. However, accumulating evidence indicates that nicotine has a neuroprotective role against nigrostriatal degeneration in rodents. Furthermore, results show that nicotine can result in an enhanced dopamine release. These observations raise the question whether the nicotine in tobacco products mediates the apparent neuroprotective effects of smoking observed in Parkinson's disease.

We have previously shown that nicotine, a predominant component of tobacco, is neuroprotective against MPP^+ toxicity in primary ventral mesencephalic cell cultures and that such neuroprotection can be prevented by pre-incubation with the non-selective nicotinic receptor antagonist d-tubocurarine. In the present study, we characterize the different nicotinic receptor populations present in mesencephalic cultures and determined the subtypes that may mediate nicotine induced neuroprotective effects. To approach this, we investigated binding of ^{3}H-epibatidine, which interacts with α2 to α6 subunit containing nicotinic receptors, and ^{125}I-α-bungarotoxin, which binds to the α7 subunit containing nicotinic receptor population. Analysis of ^{3}H-epibatidine binding best fit a two-site model in which the dissociation constants (K_D) were 0.04 and 1.2 nM and the B_{max} values were 4.7 and **6.7** fmol/10^6 cells. Saturation binding for ^{125}I-α-bungarotoxin resulted in K_D and B_{max} values of 0.8 nM and **2.8** fmol/10^6 cells, respectively.

We then sought to determine whether the α7 nicotinic receptor is involved in nicotine mediated neuroprotection. Ventral mesencephalic cell cultures were incubated with the α7 selective antagonist, α-bungarotoxin (10^{-7} M) for two hours prior to incubation with nicotine (10^{-5} M). Twenty-four hours later, cultures were exposed to 3 μM MPP^+ for 48 hours and the number of tyrosine hydroxylase immunoreactive cells was counted. Nicotine exposure rescued 15% of tyrosine hydroxylase immunoreactive cells from MPP^+ toxicity, which was not reversed by exposure to α-bungarotoxin.

These results demonstrate that nicotine can protect nigral neurons in culture against MPP^+ toxicity and that this appears to be mediated by non-α7 containing nicotinic receptors. These data may have important implications for the development of subtype selective nicotinic receptor drugs as a neuroprotective strategy in the long term management of Parkinson's disease.

I9

Highly selective degeneration in rat brain following nicotine further support evidence that fasciculus retroflexus is a weak link in brain across multiple drugs of abuse

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For a number of years this laboratory has studied degeneration in brain induced by various drugs of abuse using the de Olmos silver staining technique. We have found that when administered continuously for several days at relatively low plasma levels, a variety of drugs of abuse with strong dopaminergic actions induce degeneration in axons traveling from the lateral habenula through the sheath of fasciculus retroflexus (FR) to midbrain monoaminergic nuclei. With some of these drugs, such as cocaine, this is virtually the only degeneration induced in brain.

We now report that nicotine given continuously also selectively induces degeneration in FR, but in the other half of the tract: the cholinergic axons running from medial habenula in the core of the tract to the interpeduncular nucleus. At lower doses, this degeneration is virtually the only degeneration induced in brain by nicotine, and it can be obtained at doses of nicotine which induce plasma levels comparable to those of heavy smokers. Epibatidine induced the identical selective degeneration in FR, indicating that the list of receptor mechanisms inducing this degeneration can be simplified considerably.

FR thus appears to be a weak link in brain for diverse drugs of abuse when administered incessantly for several days, and alterations in this tract would be predicted to be especially important for the genesis of the process of nicotine addiction, withdrawal effects, and the processes underlying relapse. However, the degeneration is only in axons in FR, with cell bodies and terminals showing no signs of degeneration. This raises the interesting puzzle: what kind of mechanism would cause degeneration in only axons?

I10

Endogenous opioids suppress activation of nociceptors by sub-nanomolar nicotine

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Nicotine is known to suppress inflammatory response via neural/endocrine systems. However, when given at very high doses, it has also been found to enhance inflammation, as measured by plasma extravasation. The present study was designed to examine if low-dose nicotine has any pro-inflammatory effect.

We report that nicotine enhanced inflammatory response of the knee joint after a specific subtype (delta) of opioid receptor was blocked. These receptors appeared to be on the sensory nerve terminal and tonically activated by opioids from the adrenal medulla. This pro-inflammatory effect of nicotine was sensory nerve-dependent and was mediated by substance P, a sensory peptide. Our findings demonstrate that just like high-dose nicotine, nicotine at low-dose can enhance inflammation. This pro-inflammatory effect of low-dose nicotine, however, is masked by endogenous opioids.

Although it has been reported that some subtypes of nicotinic receptor can be activated by nicotine at doses similar to those used in this present study, the nicotinic receptor subtype involved in our study remains to be determined. We will also examine the effect of low-dose nicotine on sympathetic nerve, which co-exists with sensory nerve in the knee joint, and the possible interactions between the sympathetic and sensory nerve.

The dose of nicotine used in our study was so low that it could be found in smokers, patients taking nicotine for therapeutic purposes and in second-hand smokers. Therefore, our findings may provide important warning regarding to adverse effects of nicotine as this substance is being explored for possible comercial use as a pain-killer. In addition, since the functional interaction between nicotinic and opioidergic receptors we described in the periphery could also exist in the central nervous system, our findings may suggest an important functional relationship between these receptors.

POSTER SESSIONS
Session I: Nicotine Dependence

I11

Nicotinic excitation of nociceptive neurons in trigeminal subnucleus caudalis: desensitization and cross-desensitization of responses to strong acids and salts.

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The goal of our project is to understand the neural mechanisms underlying both the irritant and pain-reducing effects of nicotine in tobacco. Oral nicotine elicits irritation or pain in humans, and excites pain-transmitting neurons in trigeminal subnucleus caudalis (Vc) in rats. The present study further characterizes the responses of Vc neurons to nicotine and strong acid and salt stimuli.

In rats anesthetized with halothane and thiopental, single-unit recordings were made from nociceptive neurons in superficial layers of dorsomedial Vc that respond to noxious thermal and mechanical stimulation of the tongue. Nicotine (600 mM) or other irritants (5 M NaCl, 300 mM citric acid, 300 mM pentanoic acid) were separately delivered to the tongue by constant flow (0.32 ml/min) for 15 or 25 min.

Nicotine induced a significant increase in firing rate of Vc neurons within 6 min, followed by a decline back to the baseline level. This desensitization or adaptation parallels human psychophysical studies showing a decline in the perceived intensity of irritation elicited by repeated lingual application of nicotine[1]. Following a 15- or 30-min rest period, reapplication of nicotine no longer activated Vc neurons, indicative of self-desensitization. This again parallels human studies in which reapplication of nicotine elicited significantly lower intensity ratings[1]. Constant-flow application of 5 M NaCl to the tongue increased Vc neuronal firing over the initial 10 min, a pattern referred to as sensitization. In human studies, repeated application of 5 M NaCl evoked an irritant sensation that similarly grew progressively[2]. 300 mM citric acid or pentanoic acid evoked a similar pattern of sensitization in Vc neuronal responses, again paralleling human studies showing that repeated lingual application of 250 mM citric acid elicited sensitization in the perceived irritant intensity[3]. We additionally tested for nicotine cross-desensitization to acid. After recording the responses of Vc neurons to pentanoic acid, nicotine was then applied for 15-min. Reapplication of pentanoic acid elicited significantly smaller responses compared to the pre-nicotine level, indicative of cross-desensitization.

These data indicate that the responses of rat Vc neurons to oral nicotine (and other irritants) accurately reflect the human perception of irritation, supporting the use of this model to study the neural mechanisms underlying perception. Future studies will investigate interactions between nicotine and menthol at the level of Vc neurons. Our project has provided new information about the neural mechanisms of irritation induced by nicotine in tobacco smoke, with relevance to issues of second-hand smoke and to a better understanding of the sensory impact of nicotine that will aid in developing methods to help smokers quite.

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I12

Neuroendocrine responses to nicotine: a physiologic probe

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The objective of this research is to develop a quantitative method for studying the hormonal response to nicotine in humans.

Four smokers received nicotine infusions of 5, 10, and 15 µg/kg x 1 min. at the same time each morning. ACTH cortisol, human growth hormone, prolactin, arginine vasopressin, and venous nicotine levels were each measured at baseline and at 2, 5, 10, 20, 40, 60, and 90 minutes after the onset of the infusion. The hormone responses varied amongst individuals and were the most significant for the 15 µg/kg/min. nicotine dose. The timing of peak nicotine venous levels paralleled the timing for peak subjective effects, as well as increases in heart rate and blood pressure, when they occurred.

The next set of eight subjects received 8, 16 and 24 µg/kg x 1 min. nicotine infusions. The hormone response data is available on the first four of these subjects, and the data on the other four subjects are pending. Different hormonal profiles were again found. The two individuals who experienced 24 µg/kg/min nicotine most aversively, had the most distinct hormonal responses. Prolactin was elevated only in a nauseated subject. The two persons with the least endocrine reactivity had past histories of alcoholism and/or other chemical dependencies. One of these subjects wanted more nicotine, and the other developed tolerance to the subjective effects of progressive nicotine doses.
There is no report in the literature which specifies that individuals may have different hormonal response patterns to the same dose of nicotine, nor a study which quantifies these differences. By developing this methodology, we hope to probe central nervous system responsiveness to nicotine. As a result of furthering our understanding of the basis for individual differences in smoking behavior, we hope to facilitate the improved targeting of tobacco use prevention, diagnostic, and treatment strategies.

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I13

Biomarkers for Tobacco Smoke Exposure

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The objective of this project is to develop methods for measuring human exposure to toxic substances present in tobacco and tobacco smoke.

Despite advances in smoking cessation research and new pharmacotherapies for tobacco addiction, many people who want to quit are unable to do so. Consequently, there is considerable interest in "harm reduction," the possibility of reducing the harmful effects of continued tobacco use in persons who are unable to quit. Tobacco companies are developing products that are claimed to deliver lower levels of toxic substances to the user than do conventional cigarettes. Tobacco addiction treatment researchers are investigating the possibility that smokers' cigarette consumption can be reduced, for example with nicotine gum or patches, and that this may lead to reduced health risk.

A major issue is how to determine whether significant reduction in exposure to toxic substances has occurred. For example, smokers who reduce the number of cigarettes that they smoke may smoke them more intensively, extract more nicotine and other toxic substances from each cigarette, and not significantly reduce their daily intake. On the other hand, smokers using nicotine patches may inhale less smoke from their cigarettes, thereby reducing their daily exposure. Therefore, simply counting cigarettes is not reliable for estimating exposure to toxic substances. Methodology for determining intake of specific toxic substances is needed to evaluate these possibilities.

Tobacco and tobacco smoke contain numerous toxic substances (for example. about 50 carcinogens have been identified), and measuring exposure to each individually would not be practical. Our approach is to develop methods for measuring specific toxic substances, or metabolic break-down products, or other tobacco-derived substances in biologic fluids (such as blood or urine) from smokers. Concentrations of these substances, called "biomarkers" can be used as a measure of exposure.

This presentation will describe our progress in the development of new analytical methods for biomarkers of tobacco smoke intake. These include a liquid chromatography - tandem mass spectrometry (LC-MS/MS) method for measuring concentrations of solanesol, a major component of cigarette smoke "tar." We have also developed a novel method for preparing a derivative of the polycyclic aromatic hydrocarbon (PAH) metabolite 1-hydroxypyrene (1-HP), which can be used as a biomarker for a major class of carcinogens found in tobacco smoke. This derivative enhances 1-HP detection by LC-MS/MS, and should make its measurement much more practical for large-scale studies than was possible by previous methods. Furthermore, this methodology should be applicable to metabolites of other PAH as well. It is anticipated that these methods will be used by tobacco addiction treatment researchers in clinical trials to evaluate potential harm reduction, to measure toxic substance exposure in persons using new tobacco products claimed to be less harmful, and to determine toxic substance exposure in persons using low nicotine-content, potentially non-addictive cigarettes.

I14

Brain metabolic changes during cigarette craving

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Background: When cigarette smokers are attempting to quit, the sensation of craving (urge to smoke) has repeatedly been associated with relapse into usage. The goal of this study is to examine activity (in the form of glucose metabolism) in specific brain regions in heavy smokers associated with craving for cigarettes. **Method:** Twenty-one adult heavy smokers (mean- 32.4 cigarettes per day) who were otherwise healthy and not attempting abstinence underwent two ^{18}F-fluorodeoxyglucose positron emission tomography (PET) scans of the brain one-week apart in randomized order. Each subject had one PET scan while viewing a neutral (nature) video (and handling a pen) and one while viewing a video containing people smoking in a variety of settings (and handling a cigarette). Subjects were rated during these sessions for the severity of their urge to smoke, along with related symptoms, such as anxiety and depression. Nonsmoking control subjects are currently being studied for comparison. **Results:** In the heavy smokers, craving levels during the session with the smoking cue video were significantly greater than the neutral session (mean 5.0 vs. 3.7 on a 10-item 7 point analog rating scale; paired student t-test, $p < .01$). No significant differences were seen between the two sessions in anxiety or mood states. A large area of increased activity (metabolism) was seen during the craving state in a part of the brain associated with emotion and level of alertness (the perigenual/ventral anterior cingulate gyrus spanning the midline). Smaller areas of activation were seen in other parts of the brain also associated with emotion (the body and posterior portion of the cingulate gyrus, left anterior temporal lobe and left posterior parietal lobe). Regional deactivation (lower brain activity) was seen in areas of the brain associated with complex thinking (the dorsal and ventral lateral prefrontal, and lateral temporal cortices), and areas associated with vision (the occipital cortices bilaterally). **Conclusion:** Our data indicates that emotional (limbic) brain regions are activated and specific cortical regions associated with complex planning and reasoning deactivated when heavy smokers crave cigarettes. A better understanding of the brain mechanisms involved in cigarette craving may, in the future, lead to better treatments for craving and for addiction to cigarettes. Such treatments may aid addicted smokers in their attempts at smoking cessation.

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