Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution: Monell Chemical Senses Center**

2. **Reporting Period (start and end date of grant award period): 01/01/11-12/31/11**

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees): Gary K. Beauchamp, PhD**

4. **Grant Contact Person's Telephone Number: 267-519-4700**

5. **Grant SAP Number: 4100054860**

6. **Project Number and Title of Research Project: 01- Effects of Environmental Tobacco Smoke Exposure on Cough in Adolescents and Adults.**

7. **Start and End Date of Research Project: 01/01/11-12/31/11**

8. **Name of Principal Investigator for the Research Project: Paul Wise, PhD**

9. **Research Project Expenses.**

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $110,918.78

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Paul Wise	Principal Investigator	11%	$8,836.83
Julie Mennella	Co- Principal Investigator	15%	$14,680.56
Susanna Finkbeiner	Research Technician	54%	$16,622.24

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Danielle Reed	Collaborator	< 1%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Research nebulizer systems (2)	Unit required for precise, digital control of nebulized (aerosolized) capsaicin solution. Allows for controlled and repeatable dosing for improved reliability of cough threshold tests and improved subject safety. Each apparatus consists of a KoKo Digidoser plus associated fittings (DeVibiss Model 646 nebulizer vessels and Rosenthal inspiratory flow restrictor valves).	$3,195.14 per unit Total = $6390.28

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No ____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Chemosensory Cough: The Role of Smoking Status (one of four projects in a P50 clinical center grant involving researchers at Monell and the University of Pennsylvania)	X NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	Nov 2011	$1.3 Million, direct costs over 5 years (for the project in question)	$ Pending (under review)
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___x____ No_____

If yes, please describe your plans:

We have no concrete plans (beyond the proposal described under section 11(A), above. However, we do plan to submit additional proposals to government agencies as ideas from the current project develop.

12. Future of Research Project. What are the future plans for this research project?

We are currently writing a manuscript for a peer-reviewed journal and will be presenting these findings at the Annual Meeting of the Association of Chemoreception Sciences in April 2012. If we are successful in obtaining more funding, we hope to develop collaborations with clinicians and other researchers to better understand the mechanisms underlying changes in cough reflex sensitivity that occur with exposure to environmental tobacco smoke. For example, further work on the genetics of cough reflex sensitivity may identify genotypes particularly vulnerable to the effects of exposure, which could both serve as biological markers for susceptibility and provide hints regarding underlying mechanisms.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No___X_____
If yes, how many students? Please specify in the tables below:

	Undergraduate/HS	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate/HS	Masters	Pre-doc	Post-doc
Hispanic				

Non-Hispanic				
Unknown				
Total				

	Undergraduate/HS	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____x_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes__x_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The health research project allowed us to purchase two research nebulizers for precise control of inhaled aerosols. Thus, we now have a functional cough reflex laboratory that can be used for further work. Funds from the project also helped expand laboratory information technology (computers) to support personnel involved in cough research in managing research efforts and processing data.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____x_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes____x_____ No_____

If yes, please describe involvement with community groups that resulted from the research project:

The opportunity to participate in this research project was available to all parents with children regardless of race or income. As a result, some of our subjects were recruited through the WIC (Parents, Infant, and Children) programs. Participation in this research program raises awareness about the effects of smoking and second hand smoke exposure among parents from all strata of education and income and therefore is of benefit to the participants.

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project Goals and Specific Aims

Cough is a reflex that protects the lungs against noxious airborne molecules and smokers have impaired cough sensitivity, which contributes to their higher rates of respiratory illness. Parents who smoke expose their children to environmental tobacco smoke (ETS), but it is not known how ETS affects the cough sensitivity of these children. This question is especially important in Pennsylvania because the smoking rate here is in the top quintile. In our research study, we will determine whether adolescents who are exposed to ETS (because one or both parents smoke) have impaired cough sensitivity relative to children of non-smokers. The information gleaned will set the stage for investigating whether reduced sensitivity contributes to illness and early initiation of smoking during adolescence.

Experimental Design/Overview.

Each parent-child dyad were phenotyped for cough sensitivity using age-appropriate psychophysical testing, blood pressure, and obesity (height, weight, percent body fat). Saliva samples will be obtained from which DNA was isolated, purified and quantitated for alleles previously identified as important in cough reflex (e.g., the chemosensory receptor *TRPV1*[28]). Smoking history was asessed by standardized questionnaires, as described herein, to determine whether greater attenuation of cough reflex is related to more adverse health outcomes (e.g., ear infections) as well as earlier initiation with smoking.

Table 1. Schedule of Events for Days A and B for the mother and child	
Day A	**Day B**
Saliva for genetic analysis (5 min)	Carbon Monoxide Levels (2 min)
Carbon Monoxide Levels (2 min)	Anthropometry (10 min)
Anthropometry (10 min)	Cough Challenge Test (20 min)
Cough Challenge Test (20 min)	Sucrose Preference Test (10 min)
Questionnaires (10 min)	Questionnaires (10 min)
24-hour dietary recall (15 min)	24-hour Dietary Recall (15 min)

As shown in **Table 1**, each parent and child were tested on two days, separated by at least two days, and testing will occur approximately 1 hour before their next scheduled meal. Testing lasted 1.5-2 hours each day and will occur in a newly renovated testing facility designed for psychophysical testing of children and their parents. At the beginning of each test day, the parent and child were asked to breath into Vitalograph (Lenexa, KS) to obtain a measure of carbon monoxide levels. On both days (Day A and B), we used psychophysical test to determine cough thresholds for both parent and child. The techniques to measure cough

thresholds are described in detail below and well established in the literature. From 1984-2005, 4,833 subjects (4,374 adults, 459 children) underwent a capsaicin cough challenge, with no serious adverse events reported[29]. Subjects included healthy volunteers as well as patients with asthma and other respiratory conditions[29]. Capsaicin, the compound that gives chili peppers their burn, is added to many foods and some personal products. The consensus in the field is that capsaicin represents a vital component of future scientific inquiry in the field of cough[29].

I. Psychophysical Testing Procedures. These testing procedures focused on understanding the cough reflex in children and their parents.

IA. Cough Challenge. The overall procedure was to have subjects inhale ascending concentrations of an irritant (capsaicin) from a nebulizer until the subject coughs twice in immediate succession[30]. Criterion of 2 consecutive coughs is commonly used as a measure of cough threshold in the field[13, 30, 31]. This method (termed a cough challenge) has been used for decades with considerable success to study the efficacy of novel cough suppressants and to quantify cough sensitivity in both health and disease[31, 32] and capsaicin, the spicy compound in hot peppers, is one of the safest and most widely chemicals used in the field[29].

Apparatus and Stimuli. Nebulizer systems from Grace Medical (http://gracemed.net/Home_Page.html) were used to deliver brief, constant doses of aerosolized capsaicin solution (see below for more details on preparation of solutions). The device consisted of a nebulizer vessels (DeVilbiss Health Care Inc., Somerset, PA, USA) controlled by a digital dosimeter (KoKo Dosimeter, Ferraris Respiratory, Louisville, CO, USA)[6, 27]. Delivery of vapor was programmed to last 1.2 s duration. An inspiratory flow regulator valve will limit inspiratory flow rate to 0.5 l/s, regardless of inspiratory force, to deliver a constant 0.02 ml of stimulus solution (in aerosolized form) per inhalation.

Table 2. Capsaicin concentrations for cough threshold tests.

Dilution Number	Concentration (µM)
0	1000.00
1	500.00
2	250.00
3	125.00
4	62.50
5	31.25
6	15.63
7	7.81
8	3.91
9	1.95
10	0.98

Solutions were prepared by dissolving 30.5 mg capsaicin in 1 ml ethanol and 1 ml polyoxyethylenesorbitan (Tween 80) and subsequently dissolved in 8 ml sterile isotonic saline to make a stock solution of 0.01 M. The capsaicin will be pharmaceutical grade from Formosa Laboratory, the only recommended supplier by the FDA (see http://www.chpa-info.org/web/newsletter/archive/PDFs/CHPAAcuteCoughSymposiumSummary.pdf).

We will use 95% pure (190 proof) ethanol made to USP specifications (Decon Labs, Inc.; 460 Glennie Circle King of Prussia, PA 19406). We used (Pharmacopeia) Tween 80, manufactured by Fluka (purchased from Sigma-Aldrich, www.sigmaaldrich.com, product number 59924). Stock solutions were further diluted with sterile isotonic saline to make serial doubling concentrations ranging from 0.98 to 1,000 µM. see **Table 2**). Blanks were treated the same (isotonic saline with the same amount of Tween 80 and ethanol as the highest capsaicin step). The blanks do not elicit cough themselves. This range of concentrations was selected based on published work using comparable methods[27,28,31]. Solutions were dispensed daily for use in testing. Stock solutions were stored in dark amber bottles under refrigeration in a locked laboratory to which only the study personnel in Drs. Mennella's and Wise's laboratories had access.

Procedures. Following not having consumed food, beverages (except for water), or smoked for at least 1 hour (for those parents who are smokers), participants will be tested individually in rooms specifically designed for sensory testing. Subjects will be instructed to complete a normal exhalation, then bring the plastic mouth-piece attached to the nebulizer system to their lips, after which they are to inhale once. This was practiced several times (with saline as the stimulus) before the testing begins. After the inhalation, an experimenter recorded the number of coughs (expulsive, not including throat clearing) if any, and subjects were asked they felt some urge to cough (e.g., a feeling of tingling/prickling/burning in the throat), but that the sensation is not strong enough to actually make them cough (hereafter referred to as cough sensitivity threshold). Past research has shown that ratings of urge to cough increase with stimulus concentration[33], and an orderly dose-response relationship can help assess the validity of cough threshold measurements. The summary of the procedures and how threshold is determined can be found in **Table 3**. This entire procedure was repeated on the second day of testing so we could determine the reliability of the measurement.

Table 3. Summary of the cough threshold test procedure and determination of threshold

1) We start all subjects with a single inhalation of our lowest concentration of capsaicin (Step 10; 0.98 µM capsaicin; this rarely makes people cough). If this lowest concentration makes the subject cough at least twice (two cough stopping criterion), we stop the test and define 0.98 µM as the cough threshold.
2) If the lowest concentration does not make the subject cough (or if the subject coughs only once), two minutes later we present the next higher concentration of capsaicin (i.e., 1.95 µM). If the subject coughs at least twice after a single inhalation of this dose, we stop the test and define 1.95 µM as the cough threshold. If the subject does not cough at least twice, we present the next higher concentration. After each trial, we ask the subjects whether they experience a tingle/scratch/burn in the throat (cough sensitivity threshold).
3) We move up the concentration series (see **Table 2**) in this fashion, with two minutes between successive inhalations, presenting each concentration only once. As soon as a given concentration triggers at least two coughs, the test stops, that concentration is defined as cough threshold, and no higher concentration is presented.
4) If we reach our highest concentration (1000 µM), this will be presented. However, even if this highest concentration fails to trigger at least two coughs, no higher concentration will be presented (we will define the threshold as the highest step and note that this concentration failed to trigger at least two coughs).
5) Though concentration will continuously go up until the stopping criterion of two coughs is attained, blanks (samples with no capsaicin) will be given at random intervals between ascending capsaicin samples to help increase the blindness of the test.

II. Health and smoking history with a focus on respiratory health

IIA. Respiratory and Otitis Media (OM) Health History. Both parents and children were administered the same questionnaire developed by the American Thoracic Society (ATS) to gather information about their health history with a focus on respiratory illnesses[35]. The questionnaires on major respiratory symptoms included: Did the child usually have a cough apart from colds in the previous year?; Did the child usually bring up phlegm apart from colds in the previous year?; Did the child ever sound wheezy apart from colds or did he/she have an attach of wheezing that has caused him/her to be short of breast in the previous year? Did the child ever have asthma or bronchitis diagnosed by a doctor? (Asthma will be an exclusion criterion in this study.) Parents were interviewed about their child's history of ear infections (otitis media) using previously described methods[36]. Parents were asked: "Has your child ever had an ear infection or an earache? Yes/No/Don't Know"; "If yes, how many times has your child had an ear infection or earache per month/per year/in lifetime?"; "How old was your child when he/she had the first ear infection or earache?"; and "How old was your child when he/she had his/her last ear infection or earache?").

IIB. Nicotine Dependence (adults only) To evaluate nicotine self-administration behavior in smokers, subjects were given the Fagerstrom test of Nicotine dependence[37,] the Michigan Nicotine Reinforcement questionnaire[38].

IIC. National Youth Tobacco Survey (NYTS) 2009 Questionnaire (children only)[40]. The NYTS, a validated self-administered pencil and paper questionnaire, were administered to each child. Included in this survey were questions about tobacco use, exposure to environmental tobacco smoke, minors' ability to purchase or otherwise obtain tobacco products, and knowledge and attitudes about tobacco. From these data, we determined if the child had ever experimented with tobacco.

IID. Carbon Monoxide Levels. At the beginning of each test day, we measured breath carbon monoxide (CO) levels using the Vitalograph (Lenexa, KS) in adults and children. The Vitalograph BreathCO Monitor is a pocket-sized instrument which can be used to measure both alveolar concentrations and environmental levels of carbon monoxide. This display indicates parts per million (ppm) of carbon monoxide.
In addition, other health outcomes were measured.

IIE. Weight and body composition. Weight and height measurements were obtained for each subject. Body mass index (BMI; the weight in kilograms divided by the square of the height in meters) was then computed. For women, BMI was categorized as follows: 18.5 kg/m^2 to 24.9 kg/m^2 (normal weight), 25.0 kg/m^2 to 29.9 kg/m^2 (overweight), and 30.0 kg/m^2 or more (obese). For children, their BMI for age was computed and then classified in one of four categories (i.e., underweight, healthy weight, overweight or obese) using the Center for Disease Control and Prevention pediatric growth charts[41]. Total body water, fat free mass and fat were estimated by bioelectrical impedance analysis (BIA)[42] using the Quantum X instrument (RJL Systems, MI) with computational adjustments for this age group. Skinfold measurements and circumferences were obtained according to the established guidelines[43].

III. Genotyping for Cough Reflex. Saliva was obtained and genomic DNA extracted, purified and quantified (Oragene; Ontario Canada). Control genotypes were be obtained to ensure parent-child allele sharing is consistent with the report of family relationship. Genes that may play a role in chemical irritation (and in turn the cough reflex) were assessed for common variants sites, e.g., *TRPV1* is the receptor for capsaicin[44]. Variant sites were genotyped using the subject's DNA, and the correlation between nucleotide variants within these genes with the phenotypic responses of the subjects were examined. The first focus was on functional variants in chemoreceptors which respond to the irritant used here (capsaicin) and with prior association with cough in human subjects (*TRPV1* I585V and *TRPV4* P19S)[45]. Because we will be submitting an NIH grant to test more subjects, we expect over time that we will have sufficient samples sizes to determine the genetic contribution to cough reflex and how that interacts with enviromental exposure to tobacco smoke.

IV. Power and Statistical Analyses. Power calculations were conducted using effect sizes gleaned from published work on cough thresholds in adult smokers and nonsmokers measured using comparable methods[8]. Given comparable effect size, a sample of 80 subjects (forty parent-child pairings) would provide a power ($1-\beta$) of 0.80 to observe a significant difference ($\alpha=0.05$) between the smoking and non-smoking parents and between the Non-ETS Exposed and ETS Exposed children.

Genotype-phenotype effects of this magnitude here, assuming $1-\beta=0.90$ and $\alpha=0.05$. The genotype-phenotype effects may be smaller but given the practical constraints on the amount of data that can be collected during the project time line and budget, this number of subjects is a balance between power to detect large effects of the ETS exposure on the cough reflex and overall project cost.

The primary outcome measures were cough threshold. The secondary outcome measures included health history, carbon monoxide levels, genotypes, smoking history, and nicotine dependence. Data were analyzed by ANOVA/general linear models using smoking status of the parent as fixed factor in all analysis (ETS-Exposed versus Non-EST Exposed). These groups were compared for cough thresholds using age and race as covariates in all analysis. We predicted a main effect of ETS exposure, with increased cough thresholds (reduced sensitivity) for exposed children and parents. Further, if smoking affects the parents (the smokers themselves) but to a larger degree than it affects the children, then a 2-way interaction would be expected. In addition, we will test the following planned comparisons: 1) The effect of exposure for children, and 2) the effect of exposure for parents.

We computed various descriptive statistics on various secondary measures, primarily to understand the subject sample in general. In addition, key characteristics of non-exposed and exposed groups were compared to determine if the groups differed substantially in other ways besides exposure status. For quantitative traits, statistics were conducted using an ANOVA with smoking status as the fixed factor and the demographic value as the dependent variable, e.g., years of school. Post-hoc tests were conducted to assign group differences. For categorical data, non-parametric tests like Chi-Square were conducted. Criterion p-value was $p \leq 0.05$.

Finally, exploratory analyses were undertaken to understand global effects of chemoreceptor genotype on cough reflex sensitivity. At this time, due to limited sample size, ability to stratify the samples according to smoking status is limited. Detailed modeling must away larger sample sizes to be aquired when continuing funding is obtained.

Results

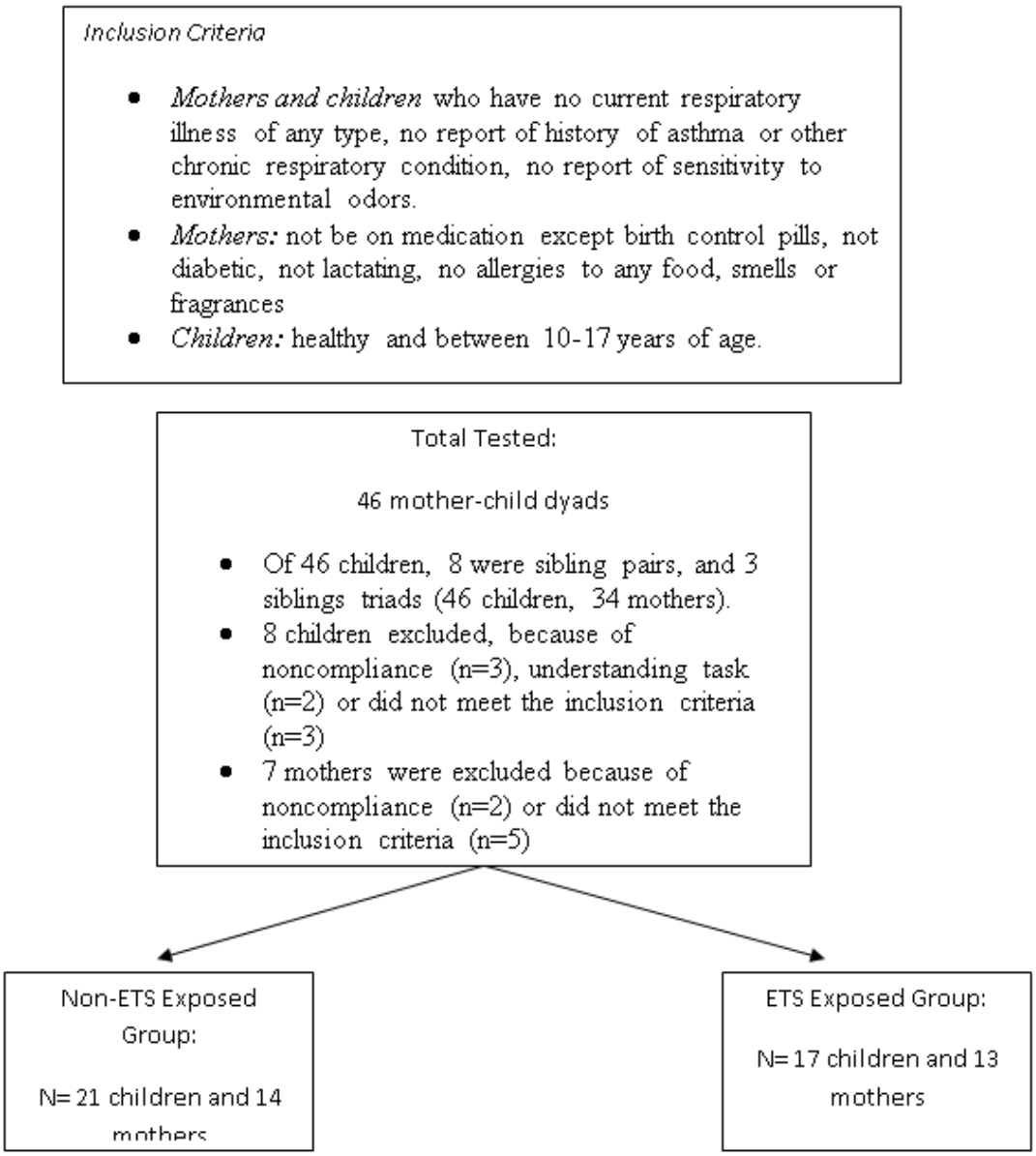
The data are presented below in the following order: a) description of subject population; b) smoking habits of mothers by self-report and subject characteristics (e.g., health characteristics) by maternal smoking status; c) cough thresholds; and d) a preliminary analysis of how genotypes for chemoreceptors are related to cough reflex sensitivity.

A) Description of Study Population

We projected that 80 subjects (including adolescents and their mothers) would be tested on two days separated by one week during the project period (January 1st 2011 to Dec 31st 2011). Mothers were recruited from newspaper advertisements, and initial interviews to screen subjects for the inclusion criteria were conducted over the telephone. Mothers were excluded from participation if they were diabetic, lactating or taking any prescription medication, with the exception of birth control pills. Children were excluded if they were less than ten or more than seventeen years of age.

Using these criteria, this goal for subject recruitment and testing was met (**Figure 1).** The total sample included 46 children and 34 mothers. The number of children tested was larger than the number of mothers because some mothers had more than one eligible child who participated in the experiment (the results on children were the primary focus of the study, so a larger sample of children was deemed acceptable). Specifically, there were 8 sibling pairs and 2 sibling triads. Of those tested, we had a sample of 21 children and 14 adults in the Non-ETS Group and 17 children and 13 adults in the ETS Group included in final analyses presented herein (see **Figure 1** for explanation; hereafter referred to as the final sample).

Figure 1. Flow diagram of children tested in the *Effects of Environmental Tobacco Smoke Exposure on Cough in Adolescents and Adults* at the Monell Center: 1/1/2011-12/31/2011



B. Description of Final Sample.

Tables 4 and **5** summarizes the demographic characteristics of this racially diverse sample of mothers and their children. On average, the mothers were 40.5 years old, had an average of 13.4 years of school (equivalent to 1.44 years of college). Some mothers reported an annual family income of less than $15,000 US dollars per year (37%) but the sample was also economically diverse, with 7.4% reporting an income of over $75,000 per year. In short, the subject sample was a reasonable sub-set of the community. African Americans were present in a slightly higher proportion than in the greater Philadelphia area, though caucasians were also well represented.

Table 4: Demographic characteristic of study population, mothers	
Number of mothers	27
Age , mean (SD)	40.5 (8.5)
Race, %, (N) % African American % Caucasian % Other/More than one race	 70.4%, (N=19) 22.2%, (N=6) 7.4%, (N=2)
Yrs of school, Mean (SD)	13.4 (2.15)
Family yearly income %<$15,000 %$15,000-$35,000 %$35,000-$75,000 %>$75,000	 37.0 %, (N=10) 29.6 %, (N=8) 25.9 %, (N=7) 7.4 %, (N=2)
Smoking status: Never-smokers Current-smoker	 51.8%, (N=14) 48.1%, (N=13)
BMI, Mean (SD)	31.2 (6.41)
Body Weight category: Normal-weight Overweight Obese	 11.1%, (N=3) 33.3%, (N=9) 55.6%, (N=15)

SD=standard deviation. Yrs=Years. Family yearly income is reported in US dollars ($). Smoking status was determined through questionnaire and confirmed with empirical measures (breath carbon monoxide concentrations). School refers to formal education, high school, community or four-year college. BMI=body mass index, a measure of obesity. BMI=kg/m^2 where kg is weight in kilograms and m is height measured in meters. For women, BMI was categorized by standards set by the Center for Disease Control (CDC): 18.5 kg/m^2 to 24.9 kg/m^2 (normal weight), 25.0 kg/m^2 to 29.9 kg/m^2 (overweight), and 30.0 kg/m^2 or more (obese).

Table 5: Demographic characteristics of children	
Number of children	38
Age, mean (SD)	13.44(2.03)
Race % African American % Caucasian % Other/More than One race)	 55.3%, N=21 34.2%, N=13 10.5%, N=4
Sex % Female % Male	 42.1%, N=16 57.9%, N=22
Yrs of school, Mean (SD)	8.13 (2.12)
By smoking status of the mother % Never-smokers % Current-smokers	 55.3%, (N=21) 44.7%, (N=17)
Body Weight category % Underweight % Healthy-weight % Overweight % Obese	 2.6%, (N=1) 63.2%,(N=24) 21.1%,(N=8) 13.2%,(N=5)
Otitis Media in life (ear infection)	YES=60.5%,(N=23), NO=39.5%, (N=15)
Frequency of ear infection in life Never 1-9 10 or more	 39.5%,(N=15) 57.9%,(N=22) 2.6%,(N=1)
Ear infection before 1 year of age Never Occasional	 73.7%, (N=28) 26.3%, (N=10)
Does child have cough when healthy?	YES=15.8%, (N=6), NO=84.2% (N=32)
Does child bring up phlegm when healthy?	YES=7.9%, (N=3), NO=92.1% (N=35)
SD=standard deviation. Yrs=Years. Smoking status was determined through questionnaire of the child's mother which was confirmed with empirical measures (breath carbon monoxide concentrations). BMI was computed for each child and then the child was classified into one of four weight categories (i.e., underweight, healthy weight, overweight or obese) using the Center for Disease Control and Prevention pediatric growth charts.	

Additional analyses involved grouping subject characteristics by ETS exposure/parent smoking status. Not all data are shown. The main point to be made is that, overall, the ETS exposed and non-exposed subjects were reasonable similar in terms of characteristics. For the mothers, never-smokers tended have more years of education, somewhat higher income, and lower diastolic blood pressure (though there was no overall difference between groups in blood pressure category **(Table 6).**

Table 6: Subject Characteristics of mothers when grouped by smoking status			
Measures	Non-ETS Group	ETS Group	p-value
N=27	N=14	N=13	
Age (Mean)(SD)	41.39(8.23)	39.61(9.06)	0.60
Years of school	14.50(2.28)	12.31(1.32)	0.01
Yearly income <$15,000 $15,000-35,000 $35,000-75,000 >$75,000	 21.43%, N=3 21.43%, N=3 50%, N=7 7.14%, N=1	 53.85%, N=7 38.46, N=5 0%, N=0 7.69%, N=1	0.03
BMI (kg/m2)	30.65(7.06)	31.88(5.84)	0.63
Waist-to-hip ratio, Mean (SD)	0.83(0.05)	0.83(0.07)	0.97
Waist circumference (cm)	94.48(14.82)	96.78(12.73)	0.67
% body fat	41.30(6.28)	39.28(5.64)	0.39
BMR (calories)	1556.86(213.18)	1654.23(234.69)	0.27
Systolic Pressure, mmHg (average of 4 measurements)	124.55(7.74)	129.46(13.43)	0.25
Diastolic Pressure, mmHg (average of 4 measurements)	72.64(7.80)	81.45(9.52)	0.01
Blood Pressure Category **Normal**, SP<120 mm and DBP Hg< 80 mmHg **At Risk**, SBP=120-139 mmHg and DBP=80-89 and mmHg **High**, >140 mmHg and >90 mmHg	• • 21.43%, N=3 • 78.57%, N=11 • 0%, N=0	 23.08%, N=3 53.85%, N=7 23.08%, N=3	0.15

SD=standard deviation. For quantitative traits, statistics were conducted using an ANOVA with smoking status as the fixed factor and the demographic value as the dependent variable, e.g., years of school. Post-hoc tests were conducted to assign group differences. For categorical data, non-parametric tests like Chi-Square were conducted. Criterion p-value is $p \leq 0.05$. BMR=basal metabolic rate as measured in calories. SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure.

The children ETS exposed and non-exposed children were also similar in characteristics (some of which appear in **Table 7**). More ETS exposed children identified as mixed race, and fewer identified as Caucasian, than in the non-exposed group. Otherwise, the samples were comparable. In addition to the data shown, though the numbers of subjects involved were too small for statistical analysis, it should also be noted that there was no difference between the ETS exposed and non-exposed groups in otitis media or phlegm. No children reported wheezing. In short, by the secondary measures collected, the two groups of children appeared to be in reasonably good and comparable (across groups) health.

Table 7. Characteristic of children grouped by smoking status of the mother			
	ETS status		
	Non-ETS Group	ETS Group	p-value
N=38	21	17	
Age (Mean±SD)	13.77(2.18)	13.04(1.83)	0.28
Sex (%) Female Male	 52.38%, (N=11) 47.62%, (N=10)	 29.41%, (N=5) 70.59%, (N=12)	0.15
Years of school	8.76(2.21)	7.35(1.77)	0.04
Race (%) African American Caucasian Other(more than one race)	 52.38%,(N=11) 47.62%,(N=10) 0%,(N=0)	 58.82%,(N=10) 17.65%,(N=3) 23.53%,(N=4)	0.02
BMI category % Underweight % Healthy Weight % Overweight % Obese	 4.76%, N=1 66.67%, N=14 23.81%, N=5 4.76%, N=1	 0%, N=0 58.82%, N=10 17.65%, N=3 23.53%, N=4	0.31
Waist-to-hip ratio	0.81(0.08)	0.80(0.06)	0.90
Waist circumference (cm)	72.08(10.77)	69.79(21.38)	0.67
% fat in body	28.68(11.15)	27.36(13.55)	0.74
BMR	1438.24(207.97)	1574.24(326.01)	0.13
Systolic Pressure (average of 4 measurements), mmHg	111.76(9.20)	115.86(8.28)	0.52
Diastolic Pressure (average of 4 measurements), mm Hg	68.02(4.53)	66.53(3.30)	0.27
Blood Pressure Category Normal: SBP and DBP <90% Pre-hyper (SBP and DBP=90-94% Hypertensive: SBP and DBP>95%	 66.67, N=14 4.76%, N=1 28.57, N=6	 81.25%, N=13 12.50%, N=2 6.25%, N=1	0.19
Smoking Habits			
Current Smokers Never Smokers	0%, N=0 100%, N=0	11.76%, N=2 88.24%, N=15	
Tried cigarette smoking* Sample size is too small for analysis	YES=9.52%, N=2	YES=11.76%, N=2	

SD=standard deviation. For quantitative traits, statistics were conducted using an ANOVA with smoking status as the fixed factor and the demographic value as the dependent variable, e.g., years of school. Post-hoc tests were conducted to assign group differences. For categorical data, non-parametric tests like Chi-Square were conducted. Criterion p-value is $p \leq 0.05$. BMR=basal metabolic rate as measured in calories.

B (continued) Smoking Habits of Mothers. Of the 27 mothers in the final sample, 13 were current smokers (ETS-exposed group) and the remaining never smoked in their lifetimes (and consequently they and their children were Non-ETS Group). A 'current smoker' was defined as someone who is currently smoking at least 5 cigarettes a day and has been smoking for more than one year whereas a 'never smoker' was defined as someone who never smoked in her lifetime. As shown in **Table 8**, there was a range of nicotine dependence in the mothers who were current smokers, with some women scoring 11 on the Fagerstrom scale (indicating extreme nicotine dependence) whereas a few women reported relatively low measures of nicotine dependence, with the average rating being 7.69. The average rating corresponds to a high level of dependence, which is consistent with the inclusion criterion. Most women reported smoking from an early age; the average age of the first cigarette was 15.38 years and the average age for starting smoking cigarettes routinely was 19.15 years.

Table 8: Nicotine dependence and smoking habits of mothers who are current smokers (N=13; ETS-Group)

Measures	Mean(SD	Range
Fagerstrom (measure of nicotine dependence) Score: 0-6: low to moderate nicotine dependence 7 and up : high nicotine dependence	7.69(2.14)	3-11
Age of first cigarette (yrs)	15.38(2.29)	12-19
Age started regular smoking (yrs)	19.15(3.98)	14-30
# Cigarettes/day	9.54(2.98)	5-17.5
#Cigarettes/day (when smoking the heaviest)	16.27(4.82)	10-25
Years smoking	20.46(9.91)	6.56-39.61
Dose (pack/years) Max Dose(pack/years)	272.91(153.35) 341.36(189.54)	62.36-534.67 78.77-629.32
Did you smoke during pregnancy with child who was tested?	YES=38.46%, N=5 NO=61.54%, N=8	

Yrs=years; Mean values are followed by standard deviations, in parenthesis.

Commensurate with the range of scores of nicotine dependence, there was a range in the number of cigarettes smoked per day, with the maximum number being 17.5. In general, people vary in how heavily they smoke over time. For instance, in periods or stress, people might smoke more or in response to health concerns (or practical limitations on smoking such as working in a non-smoking environment), they will smoke less. Therefore subjects were asked to report the number of cigarettes smoked during their period of heaviest smoking and these data suggest that on average, during this period, people smoked about 16.27 cigarettes per day. As expected from the current average age of the women (40 years old) and the average age which smoking began (19 years old), most women had been smoking for an average of 21 years, or most of their adult life (although about 61.54% of the women reported a smoking hiatus during pregnancy). When each mother's smoking history was tabulated, the average pack/years was 272.91.

C. Cough Thresholds

Cough thresholds for all mothers and children appear in **Table 9.** Both airway sensation (i.e., the lowest concentration of capsaicin that the subjects reported as causing a tingling/prickling sensation in the throat) and cough reflex threshodls (i.e., the lowest concentration of capsacin that triggered at least 2 coughs) were submitted to 3-Way ANOVAs: Generation (mothers vs. children) X Trial (experimental session 1 vs. experimental session 2) X Exposure status (smoker/ETS exposed vs. never-smoker/non-exposed). For both measures, the effect of Trial failed to reach statistical significance, which showed that the group average measurement were repeatable. The effect of Generation also failed to reach significance, which suggests that, overall, thresholds were mothers and children were comparable.

Table 9. Effect of ETS-Exposure on cough sensitivity and cough thresholds in children and their mothers

	ETS-Exposed Group		Non-ETS Exposed Group		Statistical Analysis
Measure:	Mothers	Children	Mothers	Children	
Airway Sensation Threshold (µM CAP)[1]					Generation:0.30 Trial:0.9 Group:0.02 Generation x Group:0.23
Trial 1	8.0(1.2)(2.4)	4.2(1.1)(3.8)	3.7(1.2)(8.0)	3.7(1.0)(2.6)	
Trial 2	6.3(1.4)(3.0)	5.6(1.2)(4.8)	3.6(1.3)(8.6)	3.8(1.1)(3.1)	
Cough Threshold (µM CAP)[2]					Generation:0.24 Trial:0.6 Group:<0.01 Generation x Group:0.04
Trial 1	28.5(5.1)(35.7)	19.8(4.4)(15.1)	6.8(4.9)(3.3)	9.4(4.0)(8.0)	
Trial 2	27.3(3.4)(22.8)	14.5(3.0)(10.4)	7.7(3.3)(3.9)	10.7(2.7)(7.9)	

[1]Airway sensation threshold is defined as the concentration (µM) of capsaicin that subjects first report a tingling in the throat after inhalation.
[2] Cough threshold is defined as the concentration (µM) of capsaicin that subjects coughs 2 times after inhaling.
Mean (SE)(SD)

For the current hypothesis, the most impotant effect was of Group. For both airway sensation thresholds and cough thresholds, ETS exposure/Smoking groups had higher thresholds than non-exposed/neversmoking groups. At least for the adults, these results agree well with the extant literature on smoking status: **Even for nominally healthy subjects (those who show no overt signs of clinical illness), exposure to tobaccoo smoke dulls the sensitivity of vital airway protective mechanisms.** For cough thresholds, the significant Generation X Group interaction suggests that smoking status had a larger impace on cough thresholds for smokers that for cough thresholds of children. Though the meaning of this result is currently unclear, it is consistent with

what might be expected from a dose effect (smokers would be expected to have higher levels of exposure than non-smokers exposed to their ETS).
The significant Generation X Group interaction makes the planned planned comparisons (the effect of group on children and mothers separately) important in order to verify an effect of exposure in each generation. Simple ANOVAs showed that the effect of exposure reached significance both for children, $F(1, 36)= 5.51$, $p = 0.02$, and parents, $F(1, 25)=9.3753$, $p= 0.005$. **Thus, the current results extend previous work by showing that not only does smoking impair the cough reflex in mothers who smoke, it also impairs cough sensivity in their children who are exposed to ETS in the home**. Regarding airway sensation thresholds, values tended to be higher for both tobacco smoke exposed groups, but the effect of exposure status failed to reach significance both for the children ($p = 0.20$) and parents ($p = 0.10$).

Table 10: Cough threshold stratified by genotype for all subjects

Marker	Gene	Chr	bp	Rationale	$\chi 2$	p-value
rs10845279	*TAS2R20*	12	11149711	Bitter taste	7.180395	0.03
rs12226920	*TAS2R20*	12	11150046	Bitter taste	6.466112	0.04
rs10772420	*TAS2R19*	12	11174276	Bitter taste	5.228889	0.07
rs846672	*TAS2R16*	7	122630180	Bitter taste	3.637537	0.16
rs4790522	*TRPV1*	17	3469853	Irritation	3.300000	0.19
rs1308724	*TAS2R16*	7	122625379	Bitter taste	2.753835	0.25
rs10772397	*TAS2R50*	12	11138683	Bitter taste	2.735043	0.25
rs2301699	*TRPM5*	11	2444094	General taste	2.654256	0.27
rs10246939	*TAS2R38*	7	141672604	Bitter taste	2.467825	0.29
rs1726866	*TAS2R38*	7	141672705	Bitter taste	2.051010	0.36
rs2270009	*TAS2R3*	7	141464765	Bitter taste	1.935395	0.38
rs713598	*TAS2R38*	7	141673345	Bitter taste	1.689081	0.43
rs4726600	*TAS2R39*	7	142881540	Bitter taste	1.232011	0.54
rs2234233	*TAS2R1*	5	9629529	Bitter taste	.2310021	0.63
rs1548803	*TAS2R8*	12	10959031	Bitter taste	.8572561	0.65
rs34160967	*TAS2R1*	1	6635306	Bitter taste	.8070255	0.67
rs4595035	*TAS2R60*	7	143141475	Bitter taste	.6936401	0.71
rs7827617	*TRPA1*	8	72934032	Irritation	.2876630	0.87
rs7593557	*TRPM8*	2	234863788	Irritation	.2560092	0.88
rs8065080	*TRPV1*	17	3480447	Irritation	.2496228	0.88
rs11988795	*TRPA1*	8	72949601	Irritation	.0857872	0.96

Marker refers to the unique identifier (rs or reference single nucleotide polymorphism; SNP) given to all SNPs deposited in the public database (dbSNP). Gene name refers to the protein coding transcript closest to the marker. Chr and bp refer to the position of the marker according to the most recent build of the human genome (GRCh37). Data were analyzed with a one-way Kruskal-Wallis using genotype as a fixed factor. A non-parametric method of analysis was chosen because cough thresholds were not normally distributed. No attempt was made to further stratify the sample by age (parents and children) or by smoking status because due to low power.

D. Association between cough thresholds and genes for chemosensory receptors

Due to the relatively small sample size, power is severly limited. Thus, lack of a significant association between cough thresholds and polymorphisms in genes encoding a specific receptor

cannont be viewed as solid evidence that no such association exists. Regardless, significant associations were found for genes that encode the bitter receptor protein TAS2R20 (**Table 10**). These receptors are expressed in the tongue where they detect bitter taste. However, recent research has also found bitter receptors in the airways (nasal cavity, trachea, and lungs), where they mediate airway responses (e.g., airway smooth muscle response and breathing dynamics) to xenobiotics. There is currently no evidence that the bitter receptors are involved in the cough reflex, though the current pilot results suggest that they might play a role in cough sensitivity. More detailed analyes must away further accumulation of data.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics

_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify: more than one race
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For

example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the
Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____
If yes, please describe your plans:

We are currently working on a manuscript (intended for submission to JAMA) that presents the findings on cough reflex sensitivity. We are not writing on the genetic results at this time, since the power is low. However, we are planning to present both the cough threshold data and selected results of genetic analysis at the 2012 Annual Meeting for the Association of Chemoreception Sciences (the abstracts have been accepted). The abstracts follow:

Abstract #1

The Effect of Smoking and Exposure to Tobacco Smoke on Cough Thresholds

Julie A. Mennella, PhD, Paul Wise, PhD, Susana Finkbeiner and Danielle R. Reed, PhD Monell

Abstract #2

Genetic variation in bitter receptor genes is associated with cough threshold in human subjects

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The outcome of this research is to understand the relationship between smoking and airway protective reflexes in mothers who smoke and their ETS exposed children. The information gleaned will be helpful for caregivers such as physicians, nurses and psychologists who treat nicotine addiction and craving to educate patients (smokers) regarding the risks of smoking for ETS exposed children. The finding that cough reflex sensitivity is impaired in ETS exposed children is novel, and may help explain why these children are at greater risk for respiratory and other illness. Impaired cough reflex might also make mainstream smoke more tolerable, and thereby reduce barriers to children experimenting with tobacco later in life. Further studies can explore this issue.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The major discoveries of this project were 2-fold. First, we demonstrated for first time that exposure to environmental tobacco smoke in the home impairs cough reflex sensitivity in adolescent children. This information provides further information regarding the risks smokers impose on their children by choosing to smoke in the home which can be used for the purposes of community education. Second, we demonstrated for the first time that individual differences in cough reflex sensitivity may be associated with polymorphisms in the genes that encode airway chemoreceptors (though this conclusion should be regarded as more tentative). Further work will be needed to determine the reliability of these findings.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under
this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items

a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No ___X

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Wise, Paul Morris	Associate Member, Monell Chemical Senses Center
eRA COMMONS USER NAME (credential, e.g., agency login) PaulWise	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of California, Santa Barbara	B.A.	06/93	Psychology
University of California, San Diego	M.A.	06/95	Psychology
University of California, San Diego	Ph.D.	06/00	Psychology
University of California, San Diego	Postdoctoral	07/00-06/01	Chemical Senses
Monell Chemical Senses Center, Philadelphia PA	Postdoctoral	07/01-06/04	Chemical Senses

A. Positions and Honors

Positions and Employment

2004-2010 Assistant Member, Monell Chemical Senses Center, Philadelphia PA
2010-current Associate Member, Monell Chemical Senses Center, Philadelphia PA

Honors

2005 Appointed Kraft Foods Chair of Chemosensory Psychophysics at the Monell Center
2006 Moskowitz Jacobs AchemS Award for Research Excellence in the Psychophysics of Taste and Smell

B. Publications most relevant to the project, selected from over 30 peer reviewed publications

1. Wise, P.M., Radil, T., and Wysocki, C.J. (2003). Time-intensity ratings of nasal irritation from carbon dioxide. Chemical Senses, 28, 751-760. (PMID: 14654442)
2. Wise, P.M., Radil, T. and Wysocki, C.J. (2004). Temporal integration in nasal lateralization and nasal detection of carbon dioxide. Chemical Senses, 29, 137-142. (PMID: 14977810)
3. Wise, P.M., Canty, T. and Wysocki, C.J. (2005). Temporal integration of Nasal Irritation from Ammonia at Threshold and Supra-threshold Levels. Toxicological Sciences, 87, 223-31.
4. Wise, P.M., Canty, T. and Wysocki, C.J. (2006). Temporal integration in nasal lateralization of ethanol. Chemical Senses, 31, 227-235. (PMID: 16384921)
5. Wise, P.M., Toczydlowski, S.E., and Wysocki, C.J. (2007). Temporal integration in nasal lateralization of homologous alcohols. Toxicological Sciences, 99, 254-259. (PMC2567841)
6. Wise, P.M., Hansen, J.L., Reed, D.R., and Breslin, P.A. (2007). Twin study of the heritability of recognition thresholds for sour and salty taste. Chemical Senses, 32, 749-754. (PMC2085364)
7. Wise, P.M., Miyazawa, T., Gallagher, M., Preti, G. (2007). Human odor detection of homologous carboxylic acids and their binary mixtures. Chemical Senses, 32, 475-482. (PMID: 17488748)

8. Miyazawa, T., Gallagher, M., Preti. G., and Wise, P.M. (2008). Synergistic mixture Interactions in detection of perithreshold odors by humans. Chemical Senses, 33, 363-369. (PMID: 18283044)
9. Wise, P.M., Zhao, K., and Wysocki, C.J. (2009). Dynamics of Nasal Chemesthesis. Annals of the New York Academy of Science, 1170, 206-214.
10. Wise, P.M., Toczydlowski, S.E., Zhao, K., and Wysocki, C.J. (2009). Temporal integration in nasal lateralization of homologous propionates. Inhalation Toxicology, 21, 819-827. (PMC2722918)
11. Wise, P.M., Zhao, K., and Wysocki, C.J. (2010). Dynamics of nasal irritation from pulsed, homologous alcohols. Chemical Senses 35, 823-829.
12. Wise, P.M., Eades J., Preti, G., and Wysocki, C.J. (2011). The effect of menthol vapor on sensitivity to chemical irritation. Nicotine & Tobacco Research. 35(1):109-122.
13. Wise, P.M.; Lundström, J.N., and Wysocki, C.J. (2012). Stimulus Selection for Intranasal Sensory Isolation: Eugenol Is an Irritant. Chemical Senses, in press.

C. Funding

Industry funding: Eight separate industry funded basic research projects on odor, taste, and sensory irritation (six completed, two active).

Completed Government funding

1) Effects of Environmental Tobacco Smoke Exposure on Cough in Adolescents and Adults.
Agency: State (Pennsylvania Tobacco Settlement Funds)
Aims: To determine whether exposure to second hand smoke in the home impairs the cough reflex in children.
Role: PI

2) 1R03ES013969-01A1. Temporal Integration in Nasal Lateralization
Agency: NIH (NIEHS)
Aims: funded project that examined dynamics of nasal irritation, i.e., the trade-off between time and concentration in the detection of nasal irritation.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Julie A. Mennella	Member
eRA COMMONS USER NAME (credential, e.g., agency login)	
JMennella	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Loyola University, Chicago, IL	BS	06/77	Biology
DePaul University, Chicago, IL	MS	02/81	Biology
The University of Chicago, Chicago, IL	PhD	08/88	Biopsychology
Monell Chemical Senses Center, Philadelphia, PA	Postdoc	09/88-06/90	Chemical Senses

A. Personal Statement

When I was a post-doctoral fellow conducting research on the transfer of dietary volatiles to human milk, I made a serendipitous finding that human milk was flavored after a lactating woman drank one glass of beer. This led to a systematic study of the transfer of alcohol to milk and its effects on infant nursing and mother-infant interaction, the findings of which were the lead article in the October 3rd, 1991 issue of the *New England Journal of Medicine*. I have expanded this research area to include 1) the study of the effects of tobacco on lactation; 2) the study of the effect of family history of addictions on taste preferences in adults and children; and 3) the effects of environmental tobacco smoke (ETS) exposure on health outcome as well as the emotional attachment to the odor of ETS during childhood. This research topic then became a passion for it combined my interests in lactation and child development, endocrinology, women's health and history of science, for this was an area for which there was more folklore than scientific knowledge and no established experimental paradigms. I have also made a strong commitment over the years to ensure that the information gleaned from this basic research program is communicated to need-to-know groups s (see Outreach, below).

B. Positions and Honors

Positions and Employment:

1990-1995 Assistant Member, Monell Chemical Senses Center, Philadelphia, PA
1995-1999 Associate Member, Monell Chemical Senses Center, Philadelphia, PA
1999-present Member, Monell Chemical Senses Center, Philadelphia, PA

Selected Honors and Service:

1991-1993 First recipient of the Morley R. Kare Fellowship, Monell Chemical Senses Center
1991-2008 Founding Director of the Monell Science Apprenticeship Program for High School Students and Undergraduates
1996 First recipient of the Avanelle Kirksey Lectureship Award for research excellence in maternal nutrition and infant behavior, Purdue University, West Lafayette, Indiana

1997 National Institute on Alcohol Abuse and Alcoholism, Extramural Scientific Advisory Board on Fetal Alcohol Syndrome
1997 Association for Chemoreception Sciences (AChemS) Moskowitz-Jacobs Award for research excellence in psychophysics of taste and smell
1999 Elizabeth W. Bingham Award for science mentoring, Association for Women in Science
1999-present Member, Minority and Clinical Travel Fellowship Awards Committee, AChemS
2001 L. J. Filer, Jr. Lecturer, The University of Iowa School of Medicine, Department of Pediatrics
2002-present Consultant for US Agency for International Development (USAID); Infant feeding project, Lilongwe, Malawi
2003 Invited Speaker, WHO Global Health Forum, Geneva, Switzerland
2003 Member of the NIAAA Dietary Guidelines (Moderate Drinking) Working Group
2004-present Member, Scientific Advisory Committee, Shape up America!
2005-present Chair, Taste and Flavor Working Group, Best Pharmaceuticals for Children Act, Pediatric Formulation Initiative Working Group, National Institute of Child Health and Human Development.
2006-present Member, Editorial Board, Developmental Psychobiology
2007-2009 Member, Writing Group, American Heart Association "Helping Families Implement Nutrition Recommendations"
2007-present Consultant, NIH Toolbox of Neurological and Behavioral Function: Taste and Olfaction Groups
2009 Invited Seminar Speaker: Adequate Pediatric Formulations in the 21st Century (Hot Topic Seminar); American Association of Pharmaceutical Sciences, Los Angeles, CA
2010 Panel Member, NIH Consensus Development Conference: Lactose Intolerance and Health
2010 Bring the Family Address, Association for Psychological Sciences 22nd annual convention, Boston, MA

Professional Memberships: Society for the Study of Ingestive Behavior; International Society for Developmental Psychobiology; International Society for Research in Human Milk and Lactation; Association for Psychological Sciences; American Psychological Association; Association of Chemoreception Sciences; Research Society on Alcoholism, Fetal Alcohol Spectrum Disorder Study Group.

Outreach to Need-to-Know Groups: Speaker and lecturer for continuing education courses for lactation consultants, dieticians, and physicians on various topics including the formation of food preferences, physiology of lactation, and effects of moderate drinking on lactation.

C. Selected Peer-Reviewed Publications (Selected from more than 90 peer-reviewed publications)

1. Mennella JA, Beauchamp GK. The transfer of alcohol to human milk: Effects on flavor and the infant's behavior. *New England Journal of Medicine*, 325: 981-985, 1991
2. Mennella JA, Beauchamp GK. Smoking and the flavor of breast milk- Letter. *New England Journal of Medicine* 339: 1559-60, 1998.
3. Mennella JA, Garcia PL. The child's hedonic response to the smell of alcohol: Effects of parental drinking habits. *Alcoholism: Clinical and Experimental Research* 24: 1167-1171, 2000.

4. Mennella JA, Pepino MY, Reed DR. Genetic and environmental determinants of bitter perception and sweet preferences in children and adults. *Pediatrics* 115:e216-22, 2005. PMCID: 1397914
5. Pepino MY, Mennella JA. Sucrose-induced analgesia is related to sweet preferences in children but not adults. *Pain* 119: 210-217, 2005.
6. Forestell CA, Mennella JA. Children's hedonic judgments of cigarette odor: Effects of parental smoking and maternal mood. *Psychology of Addictive Behaviors* 19: 423-432, 2005.
7. Mennella JA, Yourshaw LM, Morgan, LK. Breastfeeding and smoking: Short-term effects on infant feeding and sleep. *Pediatrics* 120:497-502, 2007. PMCID: 2277470
8. Pepino MY, Mennella JA. Effects of cigarette smoking and family history of alcoholism on sweet taste perception and food cravings in women. *Alcohol: Clinical and Experimental Research* 31:1891-9, 2007. PMCID: 2268904
9. Mennella JA, Forestell CA. Children's hedonic responses to the odors of alcoholic beverages: A window to emotions. *Alcohol* 42:249-60, 2008. PMCID: 2483837
10. Gidding SS, Lichtenstein A, Faith MS, Karpyn A, **Mennella JA**, Popkin B, Rowe J, Van Horn L, Whitsel L. Implementing American Heart Association pediatric and adult nutrition guidelines: a scientific statement from the American Heart Association Nutrition Committee of the Council on Nutrition, Physical Activity and Metabolism, Council on Cardiovascular Disease in the Young, Council on Arteriosclerosis, Thrombosis and Vascular Biology, Council on Cardiovascular Nursing, Council on Epidemiology and Prevention, and Council for High Blood Pressure Research. *Circulation* 119: 1161-1175; 2009.\
11. **Mennella JA**, Pepino, MY, Duke F, Reed DR. Age modifies the genotype-phenotype relationship for the bitter receptor *TAS2R38*. *BMC Genetics* 11:60, 2010. PMID: 20594349. PubMed - indexed for MEDLINE

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Danielle Renee Reed	Member
eRA COMMONS USER NAME (credential, e.g., agency login) DANIELLEREED	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Washington	B.S.	05/84	Psychology
Yale University	Ph.D.	05/90	Psychology
Monell Chemical Senses Center		05/90	Metabolism
University of Pennsylvania	Postdoc	05/95	Genetics

A. Personal statement.

I am particularly well suited to be a collaborator for this project because Dr. Wise, Mennella and I have an established system for managing a large collection of DNA samples from children and parents tested for taste and other phenotypes. Dr. Mennella and Wise are investigators for several of my genetically oriented projects, and I likewise serve in this capacity on her behaviorally oriented projects. Dr. Mennella and I have published papers in *Pediatrics, BMC Genetics, and Chemical Senses* (listed below) that focus on genotype and how it affects taste perception in children and adults. Dr. Wise and I have co-authored a paper (also listed below) and also have submitted an abstract based on the data collected during this project period. My laboratory is well staffed and well equipped to undertake the genetics work outlined. We currently have several instruments for genotyping, well-trained personnel to manage the flow of DNA samples, and an established track record in this area, which includes genotyping for the NIH Toolbox tests designed for taste, as well as other NIH-funded investigators. I have published numerous papers on the genetics of flavor perception and consider myself expert in this area.

B. Positions and honors.

Yale Fellowship	1984-1987
Howard Heinz Endowment Fellowship	1987-1990
NIH-PHS Award # 5-T32-CA09430	1990-1992
NIH-NRSA Award #1-F32-DK08732	1992-1994
University of Pennsylvania, Research Assistant Professor	1995-2000
Monell Chemical Senses Center, Assistant Member	2000
Monell Chemical Senses Center, Associate Member	2000
NIH, Metabolism Study Section, Temporary Member	2004
NIH, IPOD Study Section, Temporary Member	2004-2005
NIH, CADO Study Section, Member	2004-2010
Queensland Institute Medical Research, Visiting Scientist	2005; 2010
Monell Chemical Senses Center, Member	2007 to current
NIH, SCS Study Section Temporary Member	2008,2010
IFF Award for Outstanding Research	2008
NIH, CIDO Study Section, Temporary Member	2009, 2012
NIH, Shared Equipment Study Section, Temporary Member	2009-10

Korean Taste Society, Invited Speaker 2011
NIH, SCOR reviewer 2012

C. Peer-reviewed publications or manuscripts in press most relevant to the current application (selected from >100).

Reed DR, Bartoshuk LM, Duffy V, Marino S, Price RA. Propylthiouracil tasting: determination of underlying threshold distributions using maximum likelihood. Chemical Senses, 1995, 20, 529-533. PMID: 8564427

Reed DR, Bartoshuk LM, Price RA. Localization of a gene for bitter-taste perception (PROP) to human chromosome 5p15, American Journal of Human Genetics, 1999, 64, 1478-1480. PMCID: PMC1377888

Guo SW, **Reed DR**. The genetics of phenylthiocarbamide perception. Annals of Human Biology, 28, 111-142, 2001. PMID: 11293722

Duffy VB, Davidson AC, Kidd JR, Kidd KK, Speed WC, Pakstis AJ, **Reed DR**, Snyder DJ, Bartoshuk LM. Bitter receptor gene (*TAS2R38*), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. Alcoholism, Clinical and Experimental Research, 2004, 28, 1629-1637. PMCID: PMC1397913

Bufe B, Breslin PAS, Kuhn C, **Reed DR**, Tharp CD, Slack JP, Kim UK, Drayna D, Meyerhof W. The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. Current Biology, 2005, 15, 322-327. PMCID: PMC1400547

Mennella JA, Pepino Y, **Reed DR**. Genetic and environmental determinants of bitter perception and sweet preference. Pediatrics, 2005, 115, e216-e222. PMCID: PMC1397914

Hansen J, **Reed DR**, Wright M, Martin N, Breslin PAS. Heritability and genetic covariation of sensitivity to PROP, SOA, quinine HCl and caffeine. Chemical Senses, 2006, 31, 403-413. PMCID: PMC1475779

Wise P, Hansen JL, **Reed DR**, Breslin PAS. Twin study of the heritability of recognition thresholds for sour and salty taste. Chemical Senses, 2007, 32, 749-754. PMCID: PMC2085364

Reed DR. Birth of a new breed of supertaster. Chemical Senses, 2008, 33, 489-91. PMID: 1856244

Coldwell SE, Oswald TK, **Reed DR**. A marker for bone growth differs between adolescents with high vs. low sugar preference. Physiology and Behavior, 2009, 96, 574-580. PMCID: PMC2764307

Mennella, J.A., Duke, F., Pepino, M.Y., **Reed DR**. Age modifies the genotype-phenotype relationship for the bitter receptor TAS2R38 BMC Genetics, 2010, *11*, 60. PMCID: in progress.

Mennella,JA., Pepino, MY, Duke FF, **Reed DR**. Psychophysical dissection of genotype effects on human bitter perception, Chemical Senses, 2010, In progress. NIHMSID: 250589

Reed DR, Zhu G., Breslin PA, Duke FF, Henders AK, Campbell MJ, Montgomery GW, Medland SE, Martin NG, Wright MJ. The perception of quinine taste intensity is associated with common genetic variants in a bitter receptor cluster on chromosome 12. Hum Mol Genet., 2010, In progress. PMCID: 2951861

Pelchat ML, Bykowski C, Duke FF, **Reed DR**. Excretion and perception of a characteristic odor in urine after asparagus ingestion: a psychophysical and genetic study. Chemical Senses, 2010, 36, 9-17. PMCID: PMC3002398.

Knaapila A; Zhu G; Medland SE; Wysocki CJ; Montgomery GW; Martin NG; Wright MJ; **Reed DR**. A genome-wide study on the perception of the odorants androstenone and galaxolide. Chemical Senses 2012; doi: 10.1093/chemse/bjs008