Response Form for the Final Performance Review Report*

1. Name of Grantee: Monell Chemical Senses Center

2. Year of Grant: 2010 Formula Grant

A. For the <u>overall grant</u>, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an "unfavorable" rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.

Management for the overall grant at Monell is the responsibility of Monell Administration. Dr. Gary Beauchamp, Monell's Director, oversees the grant by meeting periodically with individual investigators to discuss scientific progress and results. John Tran, Monell's Administrator, oversees the budget and manages expenses to ensure that investigators stay within the established budget parameters. Erin West, Monell's Research Grants & Contracts Administrator assists John Tran in ensuring all activities are in accordance with our Grant Agreements and acts as coordinator for the project by working with investigators about deadlines, assembling required reports and reviewing reports prior to final submission.

These research projects received a rating of "favorable" and therefore no additional monitoring of investigators is needed at this time.

* Please note that for grants ending on or after July 1, 2007, grantees' Final Performance Review Reports, Response Forms, and Final Progress Reports ***will be made publicly available on the CURE Program's Web site.***

Project Number: 1086001
Project Title: Effects of Environmental Tobacco Smoke Exposure on Cough in Adolescents and Adults
Investigator: Wise, Paul

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

<u>Reviewer 1:</u>

1. The accrual of subjects is key to the success of any research. The grantee should consider additional accrual to meet the sample size as originally proposed.

<u>Response:</u>
We do indeed plan to expand our database on the effect of ETS on cough reflex sensitivity, even beyond what was originally proposed, in continuing work. Though the sample we collected was sufficient to provide a reasonable initial answer to the basic question (whether exposure to ETS affects cough reflex sensitivity), we also would like to conduct exploratory analyses on the relationship between polymorphisms in genes that encode key airway receptors and individual differences in cough sensitivity. Larger samples would prove especially desirable in this regard.

2. The analysis of genotype data in the context of mixed race must take into account racial background and the potential for variability by race, especially if the two exposure groups diverge in this characteristic. Further, it may be better to focus on interactions here, as the plausible causal mechanisms would change the slopes of the response curves much more than the raw intercept. In this instance, depending upon the frequency of the mutations in the population, the interaction effects may be more easily detected than the main effects.

<u>Response:</u>
The reviewer is correct that stratification effects are possible. As we accrue larger samples, we plan to use principle components analysis to manage stratification. Another possible option would be verification of findings using family-based studies. The points about interaction effects

are also well-taken, and we will pay particular attention to interaction effects as we accumulate more data.

Reviewer 2:
None.

Reviewer 3:
As the investigators already pointed out, a larger cohort will be needed to reach a solid conclusion. But given the amount of funding, the preliminary studies are excellent.

Response:
The comment on the effect is appreciated, and we do plan to expand our data-set by seeking resources for continuing work.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: The rating for this project is the favorable and thus no response is required in this section.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: No additional comments.

Project Number: 1086002
Project Title: Effects of Chemotherapeutic Agents on the Peripheral Taste Structure and Function
Investigator: Wang, Hong

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

<u>Reviewer 1:</u>
My only recommendation is to seek a broader, interdisciplinary approach to increase the understanding of chemotherapy-related changes in oral sensation from peripheral to central mechanisms. This broader approach would require significantly more funding, definitely beyond the parameters of the present project and funding.

<u>Response:</u>
Many chemotherapy drugs have significant side-effects on multiple organs and tissues. Their effects on food intake likely involve both peripheral and central mechanisms. This study was an initial attempt to investigate the mechanisms at the peripheral level. Future studies will employ a broader approach and extend to central mechanisms.

<u>Reviewer 2:</u>
1. The PI needs to develop skills in the analysis of behavioral data. In Specific Aim 2, it appears that t-tests were used to analyze differences between Control (PBS) versus 5-FU treatment and Control (PBS) versus PTX treatment for each concentration of each tastant on each of the three test days (1 pre- and 2 post-drug injection days). If my count (2 x 3 x 6 x 3) is correct, that's a total of over 100 t-tests. Such an extremely high number of tests will lead to a high incidence of false positives (i.e., Type 1 statistical errors). These data should have been analyzed with repeated measure analysis of variance for each tastant.

Response:
The behavioral data were also analyzed with repeated measures ANNOVA using Statistica software. The table below listed *p* values from these analyses.

Drug	Day 5			Day 6			Day 7			Day 8		
	NaCl	QHCl	Acid	Sac	Suc	IMP	NaCl	QHCl	Acid	Sac	Suc	IMP
5FU	0.011	0.682	0.563	0.056	0.011	0.613	0.740	0.473	0.340	0.007	0.008	0.507
PTX	0.337	0.592	0.186	0.125	0.061	0.625	0.002	0.359	0.011	0.690	0.659	0.943

Table 1: *p* values from repeated measures ANOVA analyses of behavioral data from days 5-8 after drug treatments. Drug-treated groups were compared with the control group for each taste compound tested on a given day. 5FU: 5-fluorouracil; PTX: paclitaxel; NaCl: sodium chloride; QHCl: quinine; Acid: citric acid; Sac: saccharin; Suc: sucrose; IMP: inosine monophosphate.

2. However, the major weakness here involves the design of the behavioral experiments of Specific Aim 2. The performance of the control (PBS) group was particularly problematic. In many instances, the control group performance was changing substantially across test days, suggesting that performance levels were not allowed to stabilize before the drug treatments were initiated. Here are some examples of control group performance:

   a. Day 6 lick ratio for 0.1 M sucrose was ~2.5 but was ~4.5 on Day 8.
   b. Day 6 lick ratio for 0.6 M sucrose was ~4.0 but was ~8.0 on Day 8.
   c. Day 6 lick ratio for 16 mM saccharin was ~3.5 but was ~7.0 on Day 8.
   d. Day 6 lick ratio for 64 mM saccharin was ~3.5 but was ~7.0 on Day 8.
   e. Day 6 lick ratio for 10 mM IMP was ~2.3 but was ~0.9 on Day 8.

   If the baseline performance of the control group is not stable across days it is impossible to evaluate the effect of a treatment (in this case, chemotherapy drugs) on that behavior. One might reasonably expect that a drug treatment would consistently either increase or decrease the lick ratio for, at least, the same concentration of a given tastant. This is not the case in the present results. For example, 5-FU is reported to significantly increase the lick ratio for each of the three concentrations of sucrose of Day 6. Yet, on Day 8, 5-FU is reported to significantly decrease the lick ratio for each of the three concentrations of sucrose. Furthermore, by looking more closely at these particular results we see that if the performance of the control group had remained stable between Days 6 and 8 then there likely would have been no influence of 5-FU on Day 8.

   These concerns all add together to indicate that the design of the behavioral experiment was inadequate. It is suggested that the researchers need to conduct more preliminary studies with normal mice to obtain parameters that afford stable performance across days. This will include, but will not be limited to, separating taste test days with water only days, perhaps testing only one tastant each day, increasing the number of water trials on taste test days, and increasing the number of mice per group.

Response:
These are reasonable concerns. The day-to-day variations were likely due to inconsistencies in food and water restrictions. We have repeated the experiment since we submitted the final report with modified method for water delivery and better controlled timing for food and water restrictions. The variations on behavioral performance were reduced across different test days for the control group. The results will be submitted for publication in the near future.

3. The work of Specific Aim 1 appears sound. However, the mice in Specific Aim 1 were non-deprived whereas the mice in Specific Aim 2 were water deprived (and hence also food deprived). Why are the mice in each specific aim on different deprivation schedules? This issue has relevance, since Experiment 1 of Specific Aim 2 was terminated early because "several 5-FU and PTX treated mice became ill and some of them died on days 11 and 12..." This raises at least two issues. First, were results from the mice that died excluded from all data analyses? One assumes they would be because their taste performance might have been disrupted for reasons unrelated to the intended effect of the drug treatment. Second, is there an interaction between deprivation (or possibly the stress induced by deprivation) and drug treatment that increases morbidity? If this is the case, then the results of Specific Aim 1 cannot readily be compared to the results of Specific Aim 2.

Response:
Specific Aim 1 was a standalone study of the effects of chemotherapy drugs on taste bud structure. The results showed that all three drugs reduced the number of Ki67-positive taste progenitor cells and increased cell death in the taste epithelium. These effects were robust and should not depend on the results from Specific Aim 2. In Specific Aim 2, we used behavioral tests as an indirect measure of taste function, as performance in these tests could also be influenced by factors other than taste function. We followed the standard water and food restriction procedures for these tests. Unfortunately, water and food restrictions were necessary in order to motivate mice to lick the taste compounds (some of them were aversive). Water and food restriction might have increased the mortality of drug treatments, but there is no evidence that short-term water and food restriction in adult mice significantly alters taste bud structure or function. Later dead mice were not excluded from the analysis of test data from days 5-8, as their licks for water were still comparable to control mice. Although there were caveats in the method, results from behavioral tests can guide us to design future experiments using more direct approaches for evaluating taste function, such as gustatory nerve recordings.

4. Following on from the previous point, one wonders why the two specific aims were not combined. Why not examine taste function in the same mice that will subsequently be examined for taste bud structure and integrity? With this approach, it would be much easier to relate disrupted functions to deficient structure.

Response:
We were still at early stages of our studies. The behavioral tests in Specific Aim 2 were carried out to test the conditions for the method and to survey taste functional changes overtime after drug treatments. Because taste buds undergo constant cell turnover and renewal, it is expected

that changes in taste function due to drug treatments should be transient. These initial tests were intended to identify the time periods when taste function was altered and then recovered. Future experiments will specifically investigate taste bud structure, taste cell renewal and differentiation at these identified time points.

Reviewer 3:

1. Strong preliminary data support the hypothesis that chemotherapeutic agents such as 5-FU, PTX and cisplatin yield untoward effects on the structural integrity of the peripheral taste system. The preliminary behavioral data is not as strong as the anatomical studies. More studies of more animals need to be done in all regards.

Response:
We agree with the recommendation. Additional behavioral tests have been done since we submitted the final report.

2. With regard to the behavioral testing, this reviewer would suggest evaluating animals only once per tastant after they complete treatment and extending the concentration range. This will likely require a reevaluation of the testing design to avoid confounds of tastant testing order. The data on treatment-induced illness, i.e., number of trials completed, and number of licks to water need to be provided.

Response: Taste bud cells turnover continuously with an average half-life of 8-12 days in rodents. Recent studies also showed that different types of taste cells have distinct lifespan: type II cells (sweet, umami, and bitter cells) turnover faster than type III cells (sour and perhaps salty). Taste progenitor/stem cells constantly replenish taste buds with newborn cells to replace dying cells. The dynamic nature of taste cell turnover and renewal make it hard to predict when taste-related behaviors would be affected by the drugs and how long these effects could last. Due to these considerations, we designed the experiment to test mice at several time points after drug treatments. The purpose was to survey behavioral changes over time and to identify the time periods when the drugs had the most effect on each taste quality and when taste function recovered.

3. With regard to the behavioral data, there is concern about the potential effect of multiple testing after treatment. This reviewer would suggest testing animals only once after they complete treatment. Further, there was no way to judge the extent to which motivation effects due to treatment-induced illness are contributing to the data presented. Do the animals complete the same number of trials in the pre- vs. post-testing sessions? Does this vary with treatment (i.e., PBS vs. agent)?

Response: As explained above, the multiple-testing experiment was to survey behavioral changes over time and to identify the time periods when the drugs had the maximum effect on each taste quality and when taste function recovered. After these time periods are identified, we can carry out further experiments to test mice within these time periods. We agree that behavioral tests cannot separate out motivation effects vs. taste effects. Gustatory nerve recording experiments may provide some answers to the question. We plan to carry out these experiments in the future.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: The rating for this project is favorable and thus no response is required in this section.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: No additional comments.