PROTOCOL CSD0905: SNUS LOCAL STUDY

Description of Statistical Methods Used

Descriptive Statistics

Descriptive statistical analyses were performed for all study endpoints. Arithmetic means, standard deviations, minimum and maximum values were reported for cigarettes per day (CPD), SNUS per day, carbon monoxide biomarkers (including exhaled carbon monoxide (ECO) and percent carboxyhemoglobin (%COHb)) at baseline (defined as time 0), and at 25' post-product use, mouth-level exposure (yield-in-use, or YIU, nicotine and "tar", on a per-cigarette and per-day basis), nicotine uptake parameters (AUC_{0-90}, baseline-adjusted AUC_{0-90}, peak nicotine concentration (C_{max}), baseline-adjusted C_{max}, and time to peak nicotine concentration), cotinine at time 0, and questionnaire responses (Fagerström Test for Nicotine Dependency, Fagerström Test for Nicotine Dependency-Smokeless Tobacco, Minnesota Nicotine Withdrawal Scale, Thermometer, Attributes, and Impact). For another measure of mouth-level exposure, snus-after-use (SAU), mean amount and percent of constituent extracted were reported. These constituents included tobacco alkaloids (nicotine, nornicotine, anatabine), benzo[a]pyrene, trace metals (Cd, Cr, Ni, Pb, As, Se), and tobacco-specific nitrosamines (or TSNAs, including NNN, NAT, NAB, NNK). For some endpoints, percent change from baseline was also computed for each time point. Product use per day was descriptively analyzed by gender subgroup and, for SNUS, additionally by variety subgroup. Medians and percent changes were reported for urinary biomarkers because data points showed evidence of skewed distribution. Particulate phase biomarkers included HPMA, SPMA, HMPMA, MHBMA, CEMA, HEMA, and thiocyanate. Vapor phase biomarkers included nicotine equivalents, AAMA, GAMA, OH-PAHs, aromatic amines, and TSNAs.

Significance Level

Statistical significance was specified at $\alpha \leq 0.05$ for all comparisons, and all references to significance are made with regard to this criterion. Nominal significance was specified as $0.05 < \alpha < 0.10$. No adjustments to the significance level for multiple comparisons were performed.

Excluded Subjects

Final analyses were based on data from the 32 participants who completed the study. Four enrolled subjects who did not complete the study were excluded from all analyses. Subject #10 withdrew prior to study due to abnormal swelling in the neck; Subject #24

was dismissed due to violating protocol by smoking immediately prior to study session; Subject #29 was dismissed due to illness; Subject #35 withdrew due to a family emergency.

Other deviations were also documented for the analysis of ECO and %COHb. Subject #14's baseline ECO was collected after the start of SNUS use during Visits 1 and 3; Subject #16's baseline ECO was collected after the start of SNUS use during visit 3; Subject #3's blood collection for COHb was taken after the start of SNUS use during Visit 3. In each of these cases, the subject's data was not included.

One subject smoked zero cigarettes on the day of butt collection for Week 4 analysis of YIU, reducing the sample size to 31.

Statistical Analysis

An analysis of variance model with repeated measures was used to assess changes across time in product use, ECO, %COHb, YIU nicotine and "tar". Dependent variables were CPD, SNUS per day, ECO at time 0, ECO at 25' post-product use, %COHb at time 0, %COHb at 25' post-product use, YIU nicotine per-cigarette and per-day and YIU "tar" per-cigarette and per-day. Visit was a fixed effect in the model. Subject was a random effect. For each ANOVA, first-order autoregressive covariance structure was specified to estimate within-subject variance. Subjects were assumed to be independent of each other. The same mixed model was also used to evaluate nicotine uptake parameters, cotinine at time 0, and FTND score comparing Week 1 to Week 4. A Wilcoxon Sign-Rank test was performed to assess within-subject changes of urinary biomarkers measured in 24-hour urine collections. Kendall's τ was used to detect increasing or decreasing trends in questionnaire responses (thermometer, attributes, impact, and Minnesota Nicotine Withdrawal Scale, or MNWS).

Area-under-the-nicotine-concentration-versus-time curve (AUC) was calculated using the trapezoidal rule. Uptake parameters were calculated using both the observed concentrations and the baseline-corrected concentrations to estimate nicotine uptake from a single UB cigarette (Visit 1) and a single Camel Snus pouch (Visit 4).

Serum Nicotine Concentration Corrections

To determine the nicotine uptake from the tobacco products used in the lab, it was necessary to estimate and subtract the levels of baseline nicotine that were expected to be cleared from the blood at each time point over the collection period.

Nicotine was assumed to follow first-order kinetics. As such, the formula for exponential decay may be used to predict the amount of nicotine remaining at each time point. Use of this formula required an estimate for nicotine half-life (t½). We estimated the half-life by fitting a regression line to the log-transformed nicotine-concentration-versus-time coordinates. The negative slope of this line is the terminal elimination constant, λ_{el}. The half-life was then derived according to the following relationship (Källén, 2008):

$$\lambda_{el} = \frac{\ln 2}{t_{1/2}} \Rightarrow t_{1/2} = \frac{\ln 2}{\lambda_{el}}$$

The empirical half-life resulting from this calculation was 131 minutes. This half-life is consistent with reported values in the literature (*e.g.*, Benowitz *et al.*, 2006).

The amount of baseline nicotine remaining from same-day tobacco usage at each time point was determined by the following decay formula, expressed in terms of nicotine half-life:

$$C_t^r = C_0 \cdot (0.5)^{t/131},$$

where

C_t^r Nicotine concentration remaining from same-day tobacco usage at time t

C_0 Nicotine concentration at time 0

t Time in minutes

Observed nicotine values were adjusted as follows:

$$C_t^{'} = C_t - C_t^r,$$

where

$C_t^{'}$ Baseline-adjusted nicotine concentration at time *t*

C_t Observed nicotine at time *t*

C_t^r Nicotine concentration from previous tobacco use remaining at time *t*

Questionnaire normalization

Questionnaire endpoints were normalized to account for participant scale-usage differences prior to testing. This normalization was achieved by using the following formula:

$$\hat{y}_{ijk} = y_{ijk} - \overline{y}_{\bullet jk} + \overline{y}_{\bullet \bullet k}$$

Where

$\hat{y}_{ijk}$ Is the adjusted questionnaire response for the ith time point, j^{th} subject, k^{th} item

y_{ijk} Is the observed response for the ith time point, j^{th} subject, k^{th} item

$\overline{y}_{\bullet jk}$ Is the mean response for the jth subject across time points for the kth item

$\overline{y}_{\bullet \bullet k}$ Is the mean response for the kth item across all j subjects and i time points

REFERENCES

1. Benowitz NL, Swan GE, Jacob P 3rd, Lessov-Schlaggar CN, Tyndale RF (2006). CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. Clin Pharmacol Ther. 80:457-467.

2. Källén, A (2008). Computational Pharmakokinetics. Chapman & Hall/CRC Biostatistics Series, Taylor & Francis Group.