

PROTOCOL CSD0914: SINGLE UNIT LOCAL STUDY: DESCRIPTION OF STATISTICAL METHODS USED

Primary Objective and Endpoints

Determine serum nicotine uptake over a three hour period following initiation of product use to clarify nicotine uptake results from modern smoke-free tobacco products in previous RJRT studies. Endpoints include:

1. Observed area-under-the-concentration-versus-time curve (AUC)
2. Baseline-adjusted AUC from time 0 to 180 minutes post-product use
3. Baseline-adjusted AUC from time 0 to 60 minutes from the current study and for previous Local Studies (Camel Strips, Camel Sticks), as originally evaluated
4. Baseline-adjusted AUC from time 0 to 90 minutes from the current study and for the previous Camel Snus Local Study, as originally evaluated
5. Re-calculation of previous Local Studies baseline-adjusted AUC using a published half-life of 120 minutes in the adjustment
6. Maximum concentration (C_{\max})
7. Maximum baseline-adjusted C_{\max}
8. Time to maximum concentration (T_{\max})
9. Duration of product use
10. Ratio of smokeless AUC to cigarette AUC, both observed and baseline-adjusted

Secondary Objectives/Endpoints

Assess tobacco abstinence symptoms prior to and at designated intervals following initiation of smoke-free tobacco product use; to assess carboxyhemoglobin levels prior to and for one hour following initiation of product use after overnight tobacco abstinence; to assess mouth-level exposure to smokeless products and cigarettes; to summarize baseline (*i.e.*, prior to in-clinic product use) serum nicotine and cotinine. Endpoints include:

1. Mouth-level exposure to nicotine, as measured by:
 - a. Amount of nicotine in Camel Strips, Camel Sticks, and Camel Orbs
 - b. Amount and percent of nicotine extracted from snus

- c. Filter tip analysis (“Yield-in-Use”) on the cigarettes
2. Carboxyhemoglobin levels prior to and for one hour following initiation of product use.
3. Tobacco abstinence symptoms (depression, anxiety, irritability, restlessness, hunger, poor concentration, and urge to smoke) as assessed by Mood and Physical Symptoms Scale (MPSS) questionnaire responses

Statistical Analysis

General Considerations

Excluded Subjects

One subject experienced an adverse event while using Camel Snus and removed the product after 7.5 minutes. Data for that subject are not included in the determinations for Camel Snus.

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. No adjustments to the significance level for multiple comparisons were performed.

Baseline Serum Nicotine and Cotinine Concentrations

Baseline nicotine was computed as the average of the -2' and 0' concentrations. For cotinine, only the -2 minute sample was considered the baseline due to the long half-life of cotinine. Both baseline nicotine and cotinine were analyzed using the following descriptive statistics: N, mean, standard deviation, minimum, and maximum.

Baseline Adjustment

Both the observed and baseline-adjusted nicotine concentrations were used for analysis. Baseline-adjusted concentrations were achieved according to the methods described below.

Current Study:

Calculate baseline nicotine as the average of the -2' and 0' samples. Subtract result from all subsequent nicotine concentrations.

Previous Studies:

For two of the three previous studies (*i.e.*, the Strips and Sticks Local Studies), we used the available 30 minutes' worth of cigarette data to compute half-life for three time intervals: 10'-15', 15'-30', and 10'-30' minutes. The resulting estimated half-lives, rounded to nearest 5', were 35' for the Strips Local Study and 30' for the Sticks Local Study. These half-lives were used to estimate and subtract the amount of nicotine remaining at each time point following initiation of product use using a model that assumes that nicotine kinetics follow first-order exponential decay. For the Snus study, additional data were available to estimate the terminal elimination rate constant using the method described in Källén, 2008. The resulting half-life using this method was 131 minutes.

Regardless of the method of half-life estimation, the model used to estimate and subtract the amount of nicotine remaining was the first-order exponential decay model expressed in terms of nicotine half-life (rather than the terminal elimination rate constant), specifically:

$$C'_t = C_t - C_0 \cdot (1/2)^{(t \div t_{1/2})}$$

Where

t = time in minutes

C'_t = Adjusted concentration at time t

C_t = Observed concentration at time t

C₀ = Nicotine concentration at time 0

t_{1/2} = estimated nicotine half-life in minutes

Assessments, Primary

Individual Nicotine Concentrations

Individual pharmacokinetic concentrations were summarized from the current study by time point, using number of observations, mean, standard deviation, minimum and maximum. Results were presented in graphical format.

Nicotine Pharmacokinetic Parameters

For each subject and product, calculate the observed (*i.e.*, using the unadjusted concentrations) and baseline-adjusted areas-under-the-curve (AUC₀₋₁₈₀ and AUC₀₋₁₈₀,

baseline-adjusted, respectively) from 0'-180' using the linear trapezoidal rule. Similarly for each subject and product, calculate the observed and baseline-adjusted maximum concentrations (C_{\max} and $C_{\max, \text{baseline-adjusted}}$, respectively) from the observed data. Calculate T_{\max} for each subject and product from the observed data based on the unadjusted concentrations. Summarize each of these parameters using N, mean, and standard deviation. Nicotine uptake parameters are further discussed by rank-ordering the products according to their mean parameter values.

The ratio of smokeless tobacco AUC to cigarette AUC was also computed, both for the observed and adjusted data, presenting as a percentage, and summarized using n, mean, standard deviation, minimum and maximum.

In-clinic Product Use Duration

In-clinic product use duration was calculated as the stop time minus the start time and expressed as minutes. Product Use Duration was summarized using these descriptive statistics: N, mean, standard deviation, minimum and maximum.

Assessments, Secondary

Mouth-Level Exposure

Mouth-level exposure (MLE) to nicotine for all study products was calculated for each subject and product. For cigarettes, MLE was evaluated using filter tip analysis (*i.e.*, "yield-in-use"). For Camel Snus, MLE is evaluated using a process referred to as snus-after-use (SAU), where both used and unused snus pouches were analyzed for nicotine content, and the difference in nicotine was calculated by subtracting the nicotine amount in used snus from the nicotine in unused pouches. The analysis accounted for the variant of Snus that the subjects used in the test session. Thus, for subjects who chose Camel Snus Frost, the SAU calculation was based on the nicotine in unused Camel Snus Frost, and similarly for Camel Snus Mellow. For Camel Sticks, Strips, and Orbs, MLE is defined as the amount of nicotine available in the product. Mean YIU and SAU were calculated and tabulated with the analytical results for Sticks, Strips, and Orbs. Additionally, individual determinations for both YIU and SAU were listed by subject.

Serum Nicotine Uptake Comparisons to Previous RJRT Studies

Baseline-adjusted AUC determinations for the current study were compared with baseline-adjusted AUC determinations for earlier RJRT studies for Snus, Sticks, and Strips. To achieve a valid comparison, the baseline-adjusted AUC_{0-60} was calculated for the current study data for comparison to the Strips and Sticks studies, and the baseline-

adjusted AUC₀₋₉₀ was calculated for the current study data for comparison to the Snus study. These results were summarized descriptively using N, mean, and standard deviation. Statistical comparisons were performed using two-sample t-tests.

Carboxyhemoglobin

Determinations of whole-blood carboxyhemoglobin percentage for each product at each time point (-2 minutes, 30 minutes, and 60 minutes) were summarized using n, mean, and standard deviation. Statistical comparisons comparing both the 30 and 60 minute determinations to the -2 minute determination were performed using paired t-tests.

Tobacco Abstinence Symptoms

The Mood and Physical Symptoms Scale (MPSS) questionnaire was administered ten times during each test visit. Subjects completed the MPSS just prior to product use, and at 5, 15, 30, 45, 60, 90, 120, 150, and 180 minutes after the start of product use.

Responses ranged from 1-5 for each symptom except for Urge to Smoke, which was rated on a scale of 0-5. Mean ratings over time are presented graphically. The mean and standard deviation for each item at each time point were also presented in tabular format.

To understand changes in rating of an MPSS item after product use, differences between the pre-product use rating and rating at each subsequent time point were averaged for each product and presented as change values in tabular format. Positive change values indicate a decrease in symptoms relative to the pre-product use rating, whereas negative change values indicate an increase in symptom. Paired t-tests were used to assess changes in rating at each time point relative to pre-product rating.

Adverse Events

Adverse effects reported by subjects and determined by the Medical Examiner as being possibly, probably, or definitely related to product use were collected and presented in tabular format by product. The total number of subjects reporting adverse events by this definition are also reported.

References

Källén, A. (2008). Computational pharmacokinetics. Boca Raton: Chapman & Hall/CRC Biostatistics Series, Taylor & Francis Group,