

ESTIMATION OF NICOTINE UPTAKE FROM TOBACCO PRODUCTS IN DUAL-USE VS. ABSTINENCE STUDY DESIGNS

Leanne C. Lee and Elaine K. Round

Research & Development, R.J. Reynolds Tobacco Company, Winston-Salem, NC 27102

ABSTRACT

R.J. Reynolds Tobacco Co. (RJRT) has explored two approaches for determining nicotine uptake from use of modern smoke-free tobacco products (MSFTs) and cigarettes in smokers: dual use and abstinence. In the dual use design, subjects smoked *ad libitum* until 30 minutes before in-clinic assessment. Subject assessment involved use of a tobacco product and timed blood collections before, during, and after product use for measurement of serum nicotine concentrations. For some MSFTs, serum nicotine concentrations decreased during and following use, indicating that the elimination rate of pre-existing nicotine from same-day smoking was greater than uptake from the MSFT. Therefore, estimation of nicotine uptake from MSFTs required adjustment for baseline nicotine from same-day smoking. To do so, it was assumed nicotine decayed following first-order kinetics, and a published nicotine half-life of 120 minutes was used. Baseline nicotine remaining at each time point was calculated and subtracted from the observed concentrations. Area-under-the-curve (AUC) was calculated using the adjusted concentrations.

In the abstinence design, smokers abstained from all nicotine-containing products, including cigarettes, for 12 hours prior to in-clinic assessment. The longer nicotine abstinence minimized baseline nicotine levels, resulting in a smaller impact of nicotine decay to the adjustment than in the dual use design. The previously described adjustment for incoming nicotine was performed, and baseline-adjusted AUCs were calculated.

Although different subject groups were employed between the two designs, results were in good agreement for all MSFTs evaluated. Cigarette AUC results from some dual use studies were lower than the corresponding AUC results from the abstinence design. Average baseline-adjusted AUCs for products ranked as follows: Cigarettes > Camel SNUS® > Camel Sticks® > Camel Strips®. The dual use design, with same-day smoking and minimal 30-minute abstinence, provided valid and accurate estimates of nicotine uptake in spite of generally larger variability.

INTRODUCTION

Early designs for acquiring a short-term, exploratory assessment of nicotine uptake of RJRT's MSFTs (Camel Strips® (Strip), Camel Sticks® (Stick), and Camel SNUS®) involved a 30-minute smoking abstinence followed by in-clinic assessment. High incoming serum nicotine levels from same-day smoking were observed and appeared to decrease following use of a single ½ Stick (Figure 1) or whole Strip. While it's possible to calculate AUC using the observed concentrations, the resulting AUC is confounded by smoking that occurred earlier in the day. A common approach for estimating nicotine uptake when background levels are present is to subtract the nicotine level at time 0 (baseline) from all nicotine concentrations and calculate AUC on the adjusted concentrations. This method is also known as net incremental AUC. However, when nicotine levels are decreasing after product use, this subtraction results in a negative AUC. Negative AUC is not possible, and it is reasonable to assume that some uptake from these MSFTs occurs. It was necessary to devise another way to account for incoming or "background" nicotine levels so that uptake from the monitored product can be calculated. The following method of background correction is described that assumes that the incoming nicotine decays following first-order kinetics. This method of correction was also applied to an abstinence study design in which the impact of background nicotine levels was minimized, thereby minimizing the background correction. The abstinence study design provides a more precise measurement of product nicotine uptake due to the low level of background nicotine.

STUDY DESIGN: Dual Use

DUAL USE: RJRT's MSFTs (Camel Strips®, Camel Sticks®, Camel SNUS®) were evaluated in three individual studies with ambulatory "migration" designs. Generally healthy male and female smokers aged 21-55 were enrolled. Subjects smoked *ad libitum* during the first week and dual-used both their usual brand (UB) cigarettes and the study-specific MSFTs for three weeks. Subjects participated in test visits, before and after the switch to dual use, during which product use occurred. Timed blood samples were collected for determination of nicotine concentrations during and following use. Subjects arrived at the testing facility following a minimum 30-minute smoking and nicotine abstinence. A catheter was placed in the antecubital region of the arm, which enabled timed blood sampling. Nicotine concentrations were measured in serum samples, and AUC was calculated to estimate nicotine uptake.

For the first blood collection of the Camel Strips® and Camel Sticks® studies, subjects smoked one UB cigarette with concurrent blood sampling for 30 minutes. At the 30 minute timepoint, subjects started use of one MSFT product with concurrent blood sampling for an additional 30 minutes. For the second blood collection three weeks later, subjects used only one Strip or approximately ½ a Stick with concurrent blood sampling for 60 minutes. Products were used until completion (Camel Strips®) or until subjects decided to end use of the product (Camel Sticks®). In the Camel SNUS® study, subjects smoked a UB cigarette during the first visit and used a Camel SNUS® pouch for up to 30 minutes during the last visit, with concurrent blood sampling lasting 90 minutes for each product.

For the first 30 minutes of product monitoring, blood samples were collected at 0, 3, 5, 7.5, 10, 15, 20, and 30 minutes. Longer monitoring periods included blood draws at every 10 minutes until monitoring was complete.

Samples were analyzed at Analytisch-biologisches Forschungslabor (München, Germany).

STUDY DESIGN: 12-Hour Tobacco Abstinence

ABSTINENCE: The abstinence study design was a crossover design, with monitoring of one product per week for three hours following the start of product use in a single test visit. A one-week washout period was utilized between products. Generally healthy males and females aged 21-55 were enrolled. The four products from the dual use studies were evaluated: subjects' UB cigarette, followed by randomly ordered Camel Strips®, Camel Sticks®, or Camel SNUS®. Subjects arrived at the testing facility approximately at 8:00 am following a minimum 12-hour smoking and nicotine abstinence intended to minimize incoming nicotine levels. A catheter was placed in the antecubital region of the forearm. Serial blood samples were collected beginning prior to product use (0 minutes) and at 3, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 minutes after the start of product use.

Samples were analyzed at Celerion (Lincoln, NE, formerly MDS Pharma).

METHODS

Visual inspection of the time-concentration profiles of the dual use study design suggested that serum nicotine decreased during MSFT use. Because subjects were using a nicotine-containing product during the assessment, nicotine concentrations were expected to increase. The likely cause for the observed decrease was nicotine decay from same-day tobacco use that occurred at a faster rate than uptake from the MSFT. Thus, in order to examine the nicotine contribution of the MSFT, nicotine from same-day cigarette usage must be uncoupled from the nicotine uptake from the MSFT.

To accomplish this, nicotine decay was assumed to follow first-order kinetics. A modified formula for exponential decay expressed in terms of nicotine half-life was used. The formula below provided background-corrected nicotine concentrations, C'_t :

$$C'_t = C_t - C_0 \cdot (0.5)^{(t \div t_{1/2})}$$

Where

C'_t	Adjusted nicotine concentrations
C_t	Observed nicotine concentrations
C_0	Nicotine concentration at time 0
t	Time in minutes
$t_{1/2}$	Published nicotine half-life, or 120 minutes (1, 3)

AUC was calculated on C'_t using the trapezoidal rule. Two-sample t-tests with unequal variance were used to compare analogous AUCs between study designs. For example, Camel Sticks® were monitored for 30 minutes and 60 minutes during the Camel Sticks® dual use study; thus, AUC from Camel Sticks® in the abstinence design was calculated as partial AUCs on both the first 30 minutes and the first 60 minutes for statistical comparison of dual use vs. abstinence Camel Sticks®.

In all cases but one, which involved the cigarette comparison, the calculated AUCs from the dual use design were not statistically significantly different from the AUCs calculated from the abstinence design (Table 1), despite generally larger variability.

Table 1. AUC Comparison

Mean (S.D.)	30-minute AUC ^a [(ng × min)/mL]		60-minute AUC [(ng × min)/mL]		90-minute AUC [(ng × min)/mL]	
	Dual Use Design	Abstinence Design	Dual Use Design	Abstinence Design	Dual Use Design	Abstinence Design
Camel Strips ^{®b}	19.0 (17.5)	13.5 (7.6)	45.3 (39.1)	32.8 (14.2)	N/A	N/A
Camel Sticks ^{®c}	24.2 (27.1)	32.2 (14.7)	88.1 (99.8)	86.6 (28.7)	N/A	N/A
Camel SNUS ^{®d}	109 (86)	102 (28)	241 (195)	222 (75)	324 (259)	319 (108)
Cigarette (Strips Study) ^e	226 (97)	294 (114)	N/A	N/A	N/A	N/A
Cigarette (Sticks Study) ^f	223 ^g (74)	294 (114)	N/A	N/A	N/A	N/A
Cigarette (SNUS Study) ^h	302 (164)	294 (114)	458 (231)	455 (172)	579 (288)	575 (214)

^a All AUCs were calculated for the interval specified using the trapezoidal rule. ^b Average estimated mouth level exposure (nicotine) = 0.54 mg/Camel Strips®. ^c Average estimated mouth level exposure (nicotine) = 1.28 mg/½ Camel Sticks®.

^d Average mouth level exposure (nicotine) = 2.28 mg/Camel SNUS® pouch. ^e Average mouth level exposure (nicotine) = 1.28 mg/cigarette. ^f Average mouth level exposure (nicotine) = 1.39 mg/cigarette. ^g Significantly different from Abstinence AUC, p = .0397. ^h Average mouth level exposure (nicotine) = 1.38 mg/cigarette.

Sample sizes: Camel Strips® Dual Use n=28, Camel Sticks® Dual Use n=27, Camel SNUS® Dual Use n=32, Abstinence Study n=15 (n=14 for Abstinence Study Camel SNUS®)

Figure 1. Serum nicotine concentrations with and without background correction (example using Camel Sticks®)

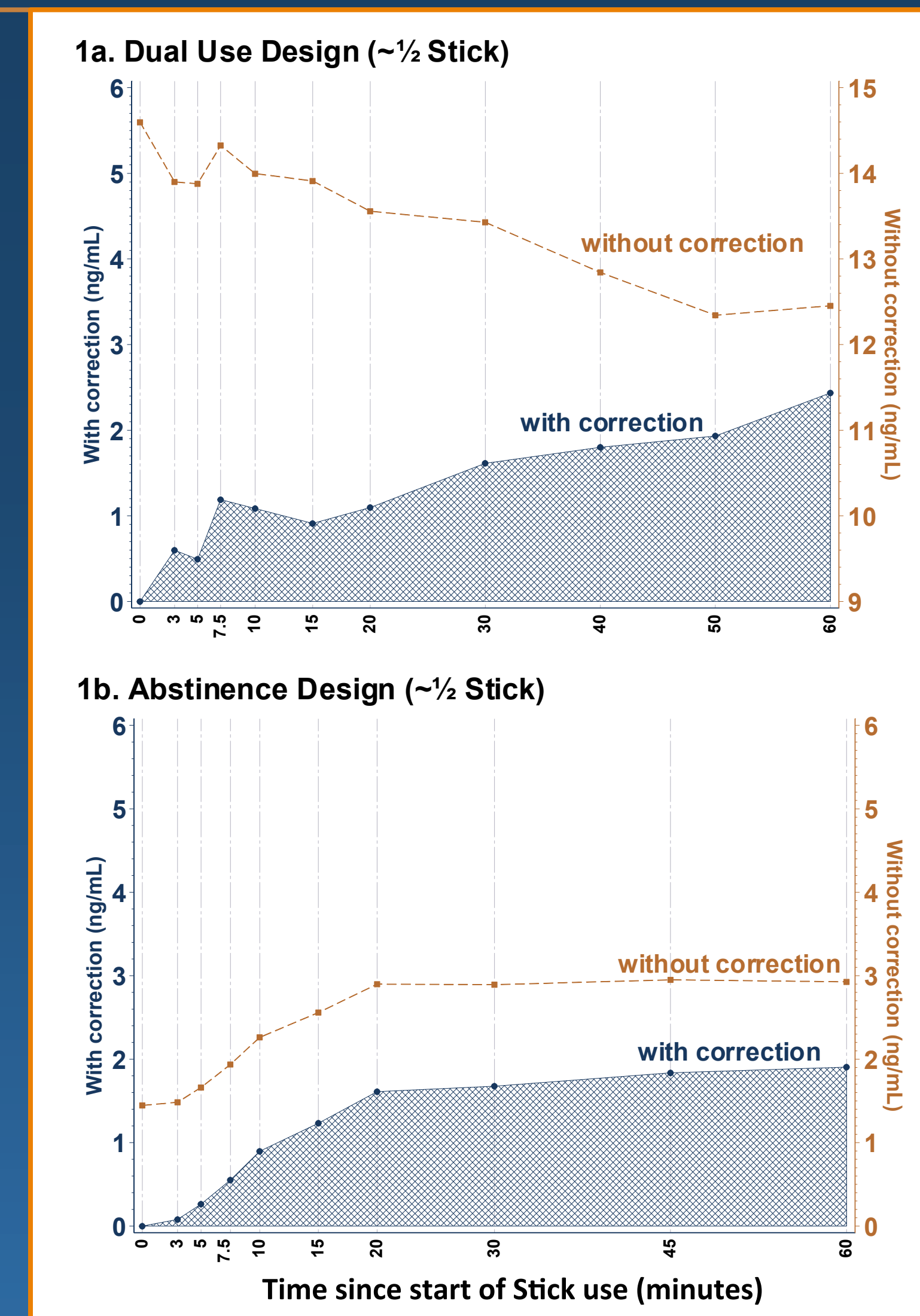


Figure 1a. In the dual use design, high mean incoming nicotine levels (14.6 ng/mL) from same-day smoking result in an apparent decrease in nicotine concentrations after Stick use (brown dashed line). After correction for incoming nicotine, blood concentrations increase. Adjusted AUC is represented by the shaded region.

Figure 1b. Uncorrected nicotine levels (brown dashed line) appear to rise after product use in the abstinence design, where incoming nicotine levels are minimal (1.4 ng/mL). The adjusted AUC (blue shaded region) is not statistically significantly different from the AUC in the dual use design.

CONCLUSION

- The background correction method adjusts nicotine concentrations for the purpose of calculating AUC. This method may be used whether or not high background levels of nicotine are present.
- Due to high incoming nicotine levels, variability was larger for the MSFTs in the dual use design. However, calculated AUCs for all MSFTs were generally not statistically significantly different between the dual use and the abstinence designs.
- While no statistically significant differences were detected, the abstinence study design is preferred for estimating nicotine exposure, because the confounding response from the cigarette is minimized.
- In terms of AUC, products ranked as follows: Cigarettes > Camel SNUS® > Camel Sticks® > Camel Strips®

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