

#### **HYPOTHESIS:**

Our hypothesis is that non-smoking adults, not exposed to environmental tobacco smoke (ETS), consuming a diet composed of foodstuffs that contain nicotine will manifest dietary exposure to nicotine by excreting in their urine both nicotine and cotinine above background concentrations.

#### **Features of diet study:**

##### **SUBJECTS:**

- a) Number - there should be at least 20 - 25 non-smoking subjects who have no smokers in their residence for any period of time. Subjects can not car-pool with persons who smoke cigarettes, pipes, or cigars.
- b) Gender - male and female subjects
- c) Age - if a single age range, then 21 - 40; if two age ranges, then 20 to 35 and 40 - 60 y.o.a.
- d) Ethnicity - Should be American by birth preferably Caucasian and Afro-American.
- e) Height and weight - no obese persons; measure for body mass index. Average height and weight.
- f) Housing - best scenario is to sequester for period ( 1 week) of diet exposure; next choice is to receive all meals on site and to wear personal monitors for ETS exposure during periods off-site. It is of extreme import to verify nicotine exposure from the diet only.

##### **DIET:**

- a) Should be a diet composed of primarily *Solanaceae*, i.e. tomatoes, potatoes, green peppers, egg plant.
- b) Beverage - either hot or iced tea, preferably Luzianne Brand of tea (instant tea / tea bags). Coffee is allowed.
- c) Mass of servings should be known and any food not eaten should be weighed, i.e. mass of consumed foods must be known for each subject.

##### **ANALYSES:**

- a) Prepared foods served to subjects including tea should be analyzed for the presence and quantification of nicotine.
- b) Types of food preparation - determine if the manner in which food is prepared affects nicotine concentration as an adjunct part of the study. May be omitted without detriment.
- c) Body fluid analyses should be done on a daily basis including a morning blood sample, (NOTE: in lieu of drawing blood, two additional saliva samples collected during the morning may be preferred) saliva samples one hour before and one hour after a meal, and 24-hour urine collections. It is preferable to analyze blood serum, saliva, and urine for the presence of nicotine and/or cotinine. Creatinine analyses should be done for all urine samples to normalize for diuresis.
- d) Each day of the study, the location of the subjects should be monitored for the presence of airborne nicotine—even in smoke-free environments—since nicotine can out-gas from smokers' clothing. The intent here is to verify exposure to nicotine only via dietary sources.

**DESIGN:**

- a) Subjects report on Monday AM to begin experiment. Subjects receive varied diet on Monday composed of foods **known to contain NO nicotine**; all body fluid samples collected.
- b) Tuesday and Wednesday - Subjects provided with a diet rich in foods **believed to contain nicotine**, prepared in a variety of ways so that palatability is not a problem.
- c) Thursday and Friday - Continue to collect body fluids for nicotine and cotinine analysis. These are "wash-out" days. No 24-hour urine collection should be begun on Friday, but whole blood and saliva samples should be taken.