

EudraCT number 2011-006110-14

Final Study Protocol

**Elevated dose of sublingual nicotine compared with Swedish snus.
Nicotine pharmacokinetics and subjective effects of single doses.**

Study Code: SM WS 12

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SIGNATURE PAGE

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Signed Agreement of the Study Protocol

We, the undersigned, have read and understood the protocol specified above, and agree on the contents. The Study Protocol and the Clinical Trial Agreement will serve as a basis for co-operation in the study.

.....

.....

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SYNOPSIS**Study code:**

SM WS 12, EudraCT number 2011-006110-14.

Title:Elevated dose of sublingual nicotine compared with Swedish snus.
Nicotine pharmacokinetics and subjective effects of single doses.**Background & rationale:**

Today there is no non-prescription drug available that can help snus users quit their habit. Results from studies of nicotine replacement therapies (NRTs) among users of smokeless tobacco products have generally been negative, in contrast to similar studies among cigarette smokers. It has been hypothesized that the lack of efficacy with NRTs among snus users may be related to differences in the nicotine delivery profile. NRTs when used as prescribed typically deliver less nicotine and at a slower rate than Swedish snus. Also, surveys have shown that 10-15% of habitual users of pouched snus products often use two pouches simultaneously, and the pinch size among users of loose snus is typically larger than the standard 1.0 g pouch. This observed usage pattern may also help to explain the lack of efficacy of NRTs among snus users. Currently available NRTs when used as prescribed may simply deliver too little nicotine to effectively reduce craving after snus cessation.

When comparing the nicotine content of different nicotine-containing products such as Swedish snus (SS) and NRT, it is important to consider that the nicotine extraction and uptake varies considerably depending on product type and formulation. With NRTs most of the nicotine is extracted and absorbed, either through the oral mucosa, or, after being mixed with saliva and swallowed, through the mucosa of the gastrointestinal tract. There it undergoes an extensive first-pass metabolism to mainly cotinine in the liver.

With snus, previous studies have indicated that on average only about 15-20% of the total nicotine content is extracted and absorbed, with large inter-individual variation. Extraction is generally not linear with pouch size: it is larger with small compared to larger pouches, which suggests that surface area, saliva penetration and diffusion factors may be more important determinants of nicotine uptake than pouch weight. Commercially available snus products have a nicotine content ranging between 1 - 2%. It is not known if the nicotine uptake from snus is linear with nicotine content.

In view of these circumstances, it is highly justified to study the nicotine delivery profile of NRTs in comparison with commercially available snus products. We have previously conducted studies of nicotine chewing gum with different nicotine content versus snus products. We now intend to extend those observations by comparing nicotine sublingual dissolvable tablets at a higher dose with Swedish snus (SS).

Primary Objective:

To compare each subject's AUC_{inf} , after administration of one single dose of 6 mg of Nicorette sublingual nicotine tablets (three 2mg tablets) to that of one single 1 g dose of SS containing 16 mg nicotine.

Secondary Objectives:

To compare plasma concentration of nicotine, T_{max} , C_{max} , AUC_t , AUC_{inf} , of one single dose of 6 mg of Nicorette sublingual nicotine tablets to 0.5

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g portion SS containing 8 mg nicotine and 1 g portion SS containing 8 mg nicotine, respectively.

To detect a difference between SS containing 8mg nicotine, 2 x 1g= total 16 mg portion snus and SS 1 g containing 16 mg nicotine.

To assess dose proportionality of nicotine after administration of one single 1 g portion of SS containing 8 and 16 mg, respectively.

To compare the *in-vivo* extracted dose of nicotine from each portion of snus, with that of the Nicorette sublingual tablet. An assumption is made that the total 6 mg amount of nicotine from the Nicorette sublingual nicotine tablets is extracted, since the tablets are completely dissolved in the mouth.

Total sample size: The study will include 16 subjects. Linear pharmacokinetics has been shown for buccal administration of nicotine in the dose interval 1-4 mg. A previous study [Lunell E & Curvall M 2011] made a calculation of sample size possible. Nicotine extraction from 1 g SS containing 8mg nicotine/pouch was estimated at 2.18 ± 0.92 mg per 1 g portion. Under the assumption of a complete dissolution and extraction of the 6mg of Nicorette sublingual nicotine tablets versus the 2.18 ± 0.92 mg nicotine, extraction levelling off above the 8mg strength for the SS and a standard deviation of 5.0 the estimated sample size is 16 with a power of 80% and $\alpha=0.05$.

Study design:

Open, randomized, five-way cross-over. Single dose administration. The Nicorette 6 mg sublingual tablets and three strengths of SS are tested. In addition two pouches of SS (8mg) will be tested. Subjects report to the laboratory for five experimental sessions. After baseline measurements, plasma nicotine concentrations are monitored over 8 hours.

Subject population:

18-50 years old, male/female (non-pregnant), healthy volunteer, using minimum 12 pouches of 1 g portion snus or half a can of loose snus per day. Female using contraceptive pill or negative pregnancy test. No smoker is allowed. Smoker is defined as "smoking during the last 24 hours according to self report and CO in exhaled air >10 ppm at clinical visits". Subjects shall be fasting overnight and abstinent from snus and all other nicotine containing products from 8.00 p.m. the night before each trial day.

Test article:

A: 6 mg dose of sublingual nicotine tablets (=3 tablets).
Batch No.:

Reference articles:

B: Swedish portion snus PSWM 0.5 g (16 mg nicotine/g)
Batch No.:

C: Swedish portion snus PSWL 1.0 g (8 mg nicotine /g)
Batch No.:

D: Swedish portion snus PSWL 1.0 g (16 mg nicotine /g)
Batch No.:

E: Swedish portion snus PSWL (8 mg nicotine /g) 2x1.0 g
Batch No.:

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Note: pH and humidity of the various types of SS will be kept constant.

Procedure:

The treatments are given as single doses in randomized order. The subject keeps the sublingual tablets still under the tongue for 30 minutes.

The subject keeps the pouch(es) of snus still between the upper lip and the gum for 30 minutes.

The subject is not allowed to eat or drink coffee and carbonated beverages or any other liquid for the first 60 minutes after dose administration [6].

Serial blood samples are drawn before, and at regular time intervals up to 6 hours after administration (13 samples). Before entry to the study subjects undergo screening evaluations including medical history, physical examination, laboratory tests and electrocardiogram.

Study parameters:

Amount of nicotine extracted, plasma nicotine concentration at 30 minutes (C_{30}), T_{max} , C_{max} , AUC_{inf} and heart rate for each treatment. The AUC_{inf} area is based on plasma data corrected for background nicotine (time zero sample).

Each subject's rating of subjective effects using a Visual Analogue Scale (VAS), anchored with "not at all" to "extremely". VAS scores will be obtained at the plasma concentrations sampling time points up to 30 minutes for:

- craving intensity
- overall "product strength" (head rush, "buzz", "hit", feeling alert)
- increased salivation
- burning sensation in the mouth and/or throat

Analysis:

The primary parameter is nicotine AUC_{inf} . The sample size calculation is based on the comparison between 6 mg of sublingual nicotine tablets, single dose, and 1 g SS (16 mg nicotine). A secondary objective is to assess dose proportionality (linear pharmacokinetics of AUC_{inf}) of nicotine after administration of one single portion of the above strengths of snus containing 8, and 16 mg nicotine, respectively. The correlation between extracted amount of nicotine and the pharmacokinetic parameters (C_{max} , T_{max} , AUC_{inf}) will also be investigated.

Assessment of dose proportionality is based on the inclusion of the 90% confidence intervals of the ratios of dose-normalized 1 g SS AUC_{inf} (16 mg nicotine), respectively, with 1 g SS AUC_{inf} (8 mg nicotine) as reference, within the interval 0.8-1.25.

The quotients (16 mg /8 mg) of AUC_{inf} and C_{max} will be analyzed with ANOVA with dose, period and subject as independent variables.

The confidence intervals will be calculated with error taken from the ANOVA. Although not used for assessment of dose proportionality also C_{max} (secondary parameter) will be tested.

Study site:

Carema Specialistvård, Eslöv.

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1. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| | |
|--------------------|--|
| AE | Adverse event |
| AUC _t | Area under the concentration versus time curve to last measurement |
| AUC _{inf} | Area under the plasma concentration versus time curve from 0 to infinity |
| β | Elimination rate constant |
| CL | Clearance |
| CL _{po} | Oral Clearance |
| C _{max} | Maximum concentration |
| CRF | Case Report Form |
| ECG | Electrocardiogram |
| HR | Heart rate |
| NRT | Nicotine Replacement Therapy |
| PK | Pharmacokinetics |
| PD | Pharmacodynamics |
| N/A | Not applicable |
| SS | Swedish snus |
| T _{max} | Time of observed C _{max} |
| T _{1/2} | Plasma nicotine half-life |

2. INTRODUCTION

a. Background

Sweden displays the lowest incidence of smoking among men in Europe. Fifty percent of snus users are ex-smokers. One explanation for the low incidence of smoking among men in Sweden may be that snus is used as a last resort for people who have failed stopping smoking with the available smoking cessation aids, such as Nicotine Replacement Therapy (NRT) products. Smokeless tobacco is capable of rapidly delivering nicotine to the bloodstream ([Fant et al 1999](#)), and therefore may be more satisfactory than e.g. NRT products.

However, approximately 1 million Swedes today use SS on a daily basis, which has raised concern among Health authorities. There is no non-prescription medicinal product for snus cessation available on the Swedish market.

b. Study rationale

Pouched SS has recently been studied in one report [[Lunell E and Curvall M 2011](#)]. The SS investigated in that report contained 8 mg nicotine and released an average 2.18 mg nicotine following use over 30 minutes. Products with higher nicotine content, a total of 16 mg, are investigated in the present study.

The rationale for the choice of the 6 mg dose of the nicotine sublingual tablet is that it proved safe in a previous study [[Molander L and Lunell E 2001](#)]. That study demonstrated a deviation from linear pharmacokinetics. The relative bioavailability of nicotine from two- and three-tablet doses was estimated at 0.82 and 0.71, respectively, compared to the one-tablet dose. A deviation from dose proportionality was thus demonstrated giving smaller than expected increase of nicotine plasma levels with increasing dose.

It was postulated that a larger fraction of the higher doses was swallowed and subject to first-pass metabolism. An analysis was therefore made on cotinine which is the main nicotine metabolite, formed to a higher extent when nicotine is swallowed. Normalized for dose, mean baseline corrected AUC_{inf} values of cotinine were estimated at 340, 364 and 404ng·h/ml following 1, 2 and 3 tablets,

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respectively. Mean cotinine/nicotine area ratios were 21.2, 26.4 and 34.8. The cotinine/nicotine ratio after three tablets was statistically significantly higher than after one tablet ($p=0.016$).

One cross-over study of steady-state nicotine plasma levels following use of four different types of SS [Lunell E and Lunell M 2005] demonstrated a similar trend towards smaller than expected increase of nicotine plasma levels with increasing dose. The 1g portion snus containing 7 mg released a similar amount of nicotine as the 0.5g portion, containing 4.5 mg nicotine. However, other factors than a higher fraction of the dose being swallowed, may explain the observed trend for snus, most important saliva penetration and diffusion factors, since the 0.5g pouch was thinner than the 1g pouch.

The present study aims at, firstly, to compare the 6 mg dose of sublingual nicotine tablets to various strengths of SS and, secondly, to investigate if there is a deviation from dose proportionality for SS containing 8mg, 16 mg, and a total of 16 mg, respectively.

The Swedish snus preparations containing higher nicotine content may not necessarily lead to a higher dose being swallowed, since snus does not stimulate salivation as strongly as such preparations as nicotine lozenge, sublingual tablet and chewing gum. On the other hand, a threshold for nicotine absorption via the oral cavity at some point seems plausible. Therefore the present study seems justified.

3 STUDY OBJECTIVES

3.1 Primary objectives

The primary objective of the present study is to compare each subject's nicotine AUC_{inf} , after administration of one single dose of 6 mg of Nicorette sublingual nicotine tablets (three 2mg tablets) to that of one single 1 g dose of SS containing 16 mg nicotine.

3.2 Secondary objectives

To compare plasma concentration of nicotine, T_{max} , C_{max} , AUC_t , AUC_{inf} , of one single dose of 6 mg of Nicorette sublingual nicotine tablets to 0.5 g portion SS containing 8 mg nicotine and 1 g portion SS containing 16 mg nicotine, respectively.

To detect a difference between SS containing 8mg nicotine, 2 x 1g= total 16 mg portion snus and SS 1 g containing 16 mg nicotine.

To assess dose proportionality of nicotine after administration of one single 1 g portion of SS containing 8 and 16 mg, respectively.

The extracted dose of nicotine from each portion of SS, will be calculated by subtracting the residual amount after use from the mean of 10 unused portions. An assumption is made that the total 6 mg amount of nicotine from the Nicorette sublingual nicotine tablets is extracted, since the tablets are completely dissolved in the mouth.

A further Secondary Objective is to compare each subject's rating of subjective effect of craving intensity, overall "product strength" (head rush, "buzz", "hit", feeling alert), increased salivation, burning sensation in the mouth and/or throat, using a 100 mm visual analogue scale (VAS) anchored with "not at all" to "extremely" at preset time points up to 30 minutes after each test product is administered.

4 INVESTIGATIONAL PLAN

Schedule of events

| EVENT | HEALTH CHECK | ECG | SAFETY LAB | VAS FORM | PK SAMPLE | AE INTERVIEW |
|-----------|--------------|-----|------------|----------|-----------|--------------|
| Screen | ✓ | ✓ | ✓ | | | |
| Predose | | | | | ✓ | ✓ |
| 2 min | | | | ✓ | ✓ | ✓ |
| 4 min | | | | ✓ | ✓ | ✓ |
| 8 min | | | | ✓ | ✓ | ✓ |
| 16 min | | | | ✓ | ✓ | ✓ |
| 24 min | | | | ✓ | ✓ | ✓ |
| 30 min | | | | ✓ | ✓ | ✓ |
| 45 min | | | | | ✓ | ✓ |
| 60 min | | | | | ✓ | ✓ |
| 1.5 hours | | | | | ✓ | ✓ |
| 2 hours | | | | | ✓ | ✓ |
| 4 hours | | | | | ✓ | ✓ |
| 6 hours | | | | | ✓ | ✓ |

STUDY OUTLINE

Open randomized single dose cross-over study on five treatments.

5.1 Selection and withdrawal of subjects

5.1.1 Inclusion criteria

1. Consent to participate voluntarily and sign Informed Consent Form prior to any study procedure.
2. Healthy male/female, age 18 through 50 years. Female using contraceptive pill or negative pregnancy test.
3. Willing and able to comply with study procedures.
4. Snus user, minimum 12 pouches snus per day of pouched portion snus, minimum 1 gram/portion or half a can of loose snus.
5. Abstinent from any form of nicotine use from 8.00 p.m. the day before each trial day.
6. Fasting overnight from 11.00 p.m.

5.1.2 Exclusion criteria

1. Smoker, defined as "smoking during the last 24 hours according to self report and CO in exhaled air >10 ppm at clinical visits"
2. Second or third degree AV block or sick sinus syndrome; congestive heart failure classified as functional Class III or IV by the New York Heart Association; myocardial infarction within six months of baseline; a prolonged QTc interval at screen or pretreatment (defined as a QTc interval of > 450 msec for males or > 470 msec for females); other clinically significant heart conditions which would negatively impact on the subject completing the study.

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3. Subjects with clinically significant liver disease which may prevent the subject from completing the study and/or an elevation in total bilirubin, alkaline phosphatase, LDH, ASAT, or ALAT of > 3 times the upper limit of the laboratory reference interval.
4. Subjects with clinically significant renal disease which may prevent the subject from completing the study and/or an elevation in serum creatinine of > 1.5 times the laboratory reference.
5. Surgery within 6 months of the Baseline visit that, in the opinion of the investigator, could negatively impact on the subject's participation in the clinical study.
6. Subjects who have participated in other drug studies within 30 days prior to enrolment.
7. Subjects with any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the drug.
8. Subjects who are using drugs capable of inducing hepatic enzyme metabolism within the previous 30 days (or 5 half lives of inducing agent, whichever is longer) of enrolment in this study.
9. Subjects with a medical history of seizures.

5.1.3 Withdrawal criteria

A subject should be withdrawn from the study treatment if, in the opinion of the Investigator, it is medically necessary, or if it is the wish of the subject.

The reason for withdrawal should be clearly described and the subject should, whenever possible, irrespective of the reason for withdrawal, as soon as possible be examined. Relevant samples should be obtained and all relevant assessments should be completed, preferably according to the schedule for the final assessment. The Case Report Form should be completed as far as possible and collected by the Monitor.

5.2 Treatments

5.2.1 Treatments administered

Test article: A: 6 mg dose of Nicorette sublingual nicotine tablets (=3 tablets).
Batch No.:

Reference articles: B: Swedish portion snus PSWM 0.5 g (16 mg nicotine/g)

Batch No.:

C: Swedish portion snus PSWL 1.0 g (8 mg nicotine /g)

Batch No.:

D: Swedish portion snus PSWL 1.0 g (16 mg nicotine /g)

Batch No.:

E: Swedish portion snus PSWL (8 mg nicotine /g) 2x1.0 g

Batch No.:

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Note: pH and humidity of the various types of SS will be kept constant.

5.2.2 Identity of investigational products

The test product, purchased from the hospital pharmacy will be delivered in its original container. Reference products will be delivered in identical food approved containers labeled with unique identification numbers.

5.2.3 Method of assigning subjects to treatment groups

Subjects will be assigned to the treatments using a computer generated randomization list.

5.2.4 Selection of doses in the study

The rationale for the choice of the 6 mg dose of the nicotine sublingual tablet is that it proved safe in a previous study [Molander L and Lunell E 2001]. Please see [section 2b](#) above.

5.2.5 Timing of dose

A single dose will be given approximately 8.00 a.m. on each study day.

5.2.6 Blinding

The present study will be a single blind study with respect to the reference products. Subjects will be administered each dose by the personnel according to the randomization list.

5.2.7 Concomitant therapy

The subjects were not allowed to eat or drink coffee and carbonated beverages or any other liquid for the first 60 minutes after dose administration [6].

Other therapy considered necessary for the subject's welfare may be given at the discretion of the Investigator. All such therapy must be recorded in the Case Report Form. No other drug under investigation may be used concomitantly with the study drug. The subjects are not allowed to participate concurrently in any other clinical study.

5.2.8 Compliance

Compliance with abstinence from smoking will be checked using an ECO (Exhaled Carbon Monoxide) measurement device, Smokelyzer. A cut-off level of ten (10) ppm will be used.

5.2.9 Emergency procedure

The Investigator is responsible for assuring that there are procedures and expertise available to cope with medical emergencies during the study.

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5.3 Pharmacokinetics

5.3.1 Sampling Procedures and analysis of used snus

The nicotine content per portion of used and unused snus, respectively, will be estimated. The mean \pm SD extracted dose of nicotine from one portion of snus, will be calculated. Nicotine is extracted from the snus using sodium hydroxide and methyl-tert-butyl ether containing quinoline as an internal standard. The nicotine present in the extract is determined by using a gas chromatograph equipped with a flame ionization detector.

5.3.2 Nicotine Concentration Measurements in plasma

Frozen plasma samples collected for nicotine determinations will be shipped to a certified contract laboratory. The analysis of nicotine in the plasma samples will be performed by LC-MS/MS at ABS Laboratories Ltd, UK.

To quantify nicotine a multilevel calibration at seven concentrations will be performed. The calibration line will be fitted by means of a power curve fitting regression model using the equation $y=ax^b$. The samples will be assayed once. If the sample shows concentrations considered by the Study Director to be outside those expected the sample will be re-assayed. If the repeat assay gives a result greater than $\pm 10\%$ of the first result a third analysis will be performed, subject to the availability of sample. The precision of the method above the 0.7 ng/ml level of nicotine is better than 12% C.V. and above 4 ng/ml better than 6% C.V. The level of quantification is 0.5 ng/ml.

An analysis will be made on cotinine which is the main nicotine metabolite, to investigate if it is formed to a higher extent from the preparations with higher nicotine content. This will be the case if a higher fraction of nicotine is swallowed. Cotinine plasma concentrations are about 10 times those of nicotine.

5.3.3 Pharmacokinetic assessments

WinNonlin computer program ([Siphar Corp., USA](#)) will be used for all pharmacokinetic calculations. Nicotine plasma concentrations are determined at preset time points, before (0), 2, 4, 8, 16, 24, 30, 45, 60 minutes, 1.5, 2, 4 and 6 hours after administration.

- T_{max}

- C_{max}

- AUC_{inf}

- $T_{1/2}$

5.3.4 Pharmacokinetic calculations

The following pharmacokinetic parameters will be measured:

Nicotine plasma concentrations

C_{max} - measured plasma peak concentration

The following pharmacokinetic parameters will be calculated:

β The elimination rate constant is obtained by linear regression analysis of the slope of the terminal part of the logarithmic plasma concentration-time curve. The individually calculated elimination rate constant is used for correction of baseline nicotine concentration.

AUC_{inf} will be calculated from the equation:

$$AUC_{inf} = AUC_{0-8} + C_{(8)} / \beta$$

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where $C_{(s)}$ denotes the last value on the concentration-time curve. AUC_{0-s} will be calculated by the trapezoidal rule.

- Calculations of dose proportionality will be performed according to Section 6.2 below.
- The cotinine/nicotine AUC ratio for each preparation will be calculated.

5.4 Safety and Efficacy variables

5.4.1 Adverse Event (AE)

An Adverse Event (AE) is any untoward medical occurrence in a subject or trial subject to whom a drug is administered or in whom a medical device is used: The event does not necessarily have a causal relationship with that treatment or usage.

Adverse Events include the following:

- a) All suspected adverse medication reactions.
- b) Apparently unrelated illnesses, including the worsening of a pre-existing illness (see 'Pre-existing Conditions' below).
- c) Injury or accidents.
- d) Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with a clinical event already reported. Laboratory abnormalities associated with a clinical event (e.g. elevated liver enzymes in a subject with jaundice) should be described under 'Comments' on the report of the clinical event rather than be listed as a separate adverse event.

5.4.2.1 Pre-existing conditions

In this trial, a pre-existing condition (i.e. a disorder present before the AE reporting period started and will be noted on the pre-treatment medical history/physical examination form) should not be reported as an AE unless the condition worsens or episodes increase in frequency during the AE reporting period.

5.4.2.2 Procedures

Diagnostic and therapeutic invasive and non-invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as the AE and the resulting appendectomy be noted under 'Comments'.

5.4.2.3 Symptoms of Original or Targeted Disease

Indicate in the AE section whether such symptoms are to be reported as AEs or not. If symptoms of the original or targeted disease are not to be considered as AEs in this trial, provide information on how they are to be reported instead: E.g. specify the appropriate sections of the CRF.

5.4.3 Serious Adverse Event (SAE)

An AE that meets one or more of the following criteria is classified as serious:

Death

Life-threatening (i.e. immediate risk of death)

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In-subject hospitalisation or prolongation of existing hospitalisation

Permanent or significant impairment of function or permanent damage to a body structure or intervention is required to prevent permanent impairment or damage

Cancer

- Congenital anomaly/birth defect
- Any other AE that the investigator or company judges to be serious, or which is defined as serious by the regulatory agency in the country in which the adverse event occurred.

5.4.4 Reporting Adverse Events

The investigator is to report all Adverse Events directly observed and all events spontaneously reported by the trial subject. In addition, each trial subject will be questioned about AEs *at each clinic visit following initiation of treatment*. Alternatively, a check list of specified symptoms may be used.

Minimum information required include:

Time of AE (start and stop dates), severity, seriousness and action taken.

The AE reporting period for this trial begins upon receiving *the first dose of investigational medication/product* (or immediately prior to the first dose of investigational medication/product, etc.) and ends in a pre-defined period after stopping trial medication, e.g. *at the final clinic visit*.

All AEs that occur in trial subjects during the AE reporting period must be reported to CROel AB whether or not the event is considered medication/product-related

In addition, any known untoward event that occurs subsequent to the AE reporting period that the investigator assesses as possibly related to the investigational medication/product should also be reported as an adverse event.

If a serious AE occurs, CROel AB is to be notified by the investigator on the Serious Adverse Event Report form within 24 hours of awareness of the event.

The initial report is to be followed by submission of more detailed and additional AE information within 5 working days of the event using the same form. If unexpected, SAEs are also to be reported immediately to the responsible Institutional Review Board/Independent Ethics Committee.

If the AE is serious, possibly related, and unexpected, the treatment code has to be broken. This is performed by CROel AB after approval by the Study Director.

SAEs should also be reported on the clinical trial CRF AE form.

Non-serious AEs are to be reported on the CRF AE forms, which are to be submitted to CROel AB as specified in the adverse event report submission procedure for this protocol.

All AEs should be followed until they are resolved, or the subject's participation in the trial ends. Instructions for reporting changes in an ongoing AE during a subject's participation in the trial are provided in the instructions that accompany the CRF AE forms.

In addition, *all serious AEs and those non-serious events assessed by the investigator as possibly related to the investigational medication/product* should continue to be followed even after the subject's participation in the trial is over. Such events should be followed until they are resolved or until the Investigator assesses them as "chronic" or "stable". Resolution of such events is to be documented on the appropriate CRF.

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6 Efficacy Assessments

Subjective effects — Each subject's rating of product "strength" using a Visual Analogue Scale (VAS), anchored with "not at all" to "extremely". VAS scores will be obtained at the same time points as the nicotine plasma concentrations sampling time points, i.e. before (0), 2, 4, 8, 16, 24 and 30 minutes after each product is administered:

- craving intensity
- overall "product strength" (head rush, "buzz", "hit", feeling alert)
- increased salivation
- burning sensation in the mouth and/or throat

7 Data Quality Assurance

Monitoring visits and a quality assurance audit to the trial site will be made during the trial, to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of agreement with data on Case Report Forms.

8 Curriculum vitae and other documents

Curriculum vitae will be collected from all personnel and be kept in the Study Master File.

9 Staff Information

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed.

10 Case Report Forms

Case Report Forms (CRFs) of a design mutually agreed upon by the Investigator and the Sponsor will be supplied by CROel AB. A CRF is required and should be completed for each included subject, and signed. Corrections of data should be made using a single line, leaving the corrected data clearly visible. The accurate data should be entered next to the inaccurate data. All changes should be initialled and dated by the Investigator. If corrections are performed by another member of the staff, the Investigator has to approve the correction. Correction fluids are not allowed.

The completed original CRFs are the sole property of CROel AB and should not be made available in any form to third parties, except for authorised representatives of appropriate Health Authorities, without written permission from the Sponsor.

11 Monitoring

The study site will be visited periodically during the study, as agreed with the Investigator. The Monitor will ensure that all aspects of the protocol are followed, including the randomisation procedure, the accurate recording of results, the reporting of Adverse Events, Product Accountability and record keeping.

Furthermore, it will be verified that the clinical facilities remain accurate, and that the Case Report Forms are in agreement with source data. For this purpose the Monitor will be given access to hospital records, original laboratory data, etc., as far as they relate to the study,

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without jeopardising subject integrity and as agreed with the Investigator prior to the study. Case Report Forms for all included subjects will be made available to the Monitor for review and collection as agreed with the Investigator. It is important that the Investigator and other relevant personnel are available during the monitoring visits and possible audits and that sufficient time is devoted to the process.

12 Record Retention

To enable any further evaluations and/or audits from Health Authorities/the Sponsor, the Investigator agrees to keep records, including the identity of all participating subjects, all original signed Informed Consent Forms, copies of all CRFs and detailed records of drug disposition. To comply with international regulations, the records should be retained by the Investigator for 15 years.

13 STATISTICAL METHODS

Statistical and analytical plan

The following is an outline of the statistical methodology that will be used to analyze this study. A more detailed description will be provided in a separate statistical analysis plan (SAP) which may also include additional exploratory analyses not explicitly mentioned in the following sections. The SAP will be finalized before closure of the study database and deviations from the SAP will be reported and justified in the clinical study report.

The primary endpoint nicotine extraction will be analyzed using student's t-test for paired observations (for the comparison between the 8 mg SS and the 6mg of Nicorette). Since this is a 5 period cross-over design no attempts will be made to correct for neither period nor carry-over effects. It is assumed that no period and carry-over effects will be presented due to the short half-life of these products.

All analyses on the secondary endpoints will be performed using either student's t-test for continuous variables or the Mantel-Haenszel chi square for dichotomous variables.

All analyses will be performed using a 5% significance level and two sided tests.

Pharmacokinetics

Plasma samples drawn at regular intervals for up to 6 hours after dose administration will be analyzed for nicotine. From the time-concentration curves the T_{max} , C_{max} , AUC_t , AUC_{inf} and $T_{1/2}$ will be calculated.

The primary objective of the present study is to compare each subject's nicotine AUC_{inf} after administration of one single dose of 6 mg of Nicorette sublingual nicotine tablets (three 2mg tablets) to that of one single 1 g dose of SS containing 16 mg nicotine.

A secondary objective is to compare plasma concentration of nicotine, T_{max} , C_{max} , AUC_t , AUC_{inf} , of the 6 mg dose of Nicorette sublingual nicotine tablets to 0.5 g portion SS containing 8 mg nicotine and 1 g portion SS containing 16 mg nicotine, respectively.

A secondary objective is also to detect a difference between SS containing 8mg nicotine, 2 x 1g portion snus and SS containing 16 mg nicotine.

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A further secondary objective to assess dose proportionality of nicotine after administration of one single portion of 1 g SS containing 8mg, and one single portion of 1 g SS containing 16 mg, respectively.

Dose proportionality will be based on AUC_{inf} and assessed as follows:

$$F_{rel} = \frac{\frac{AUC_{16\text{ mg}}}{16/8}}{AUC_{8\text{ mg}}}$$

A 90% confidence interval of geometric mean F_{rel} within 0.8-1.25 indicates linear pharmacokinetics.

Calculations of dose proportionality will be performed on plasma values corrected for background nicotine (time zero concentration). AUC_{inf} corrected for baseline nicotine concentration (C_0) is calculated according to equation

$$cAUC_{inf} = AUC_{inf} - C_0 / \beta$$

The primary parameter is AUC_{inf} . Although not used for assessment of dose proportionality also C_{max} (secondary parameter) will be tested for dose linearity.

Assessment of dose proportionality is based on the inclusion of the 90% confidence intervals of the ratios of dose-normalized AUC_{inf} (16 mg) and AUC_{inf} (16 mg), respectively, with AUC_{inf} (8 mg) as reference, within the interval 0.8-1.25. AUC_{inf} and C_{max} will be normalized for dose, log-transformed and tested for log-normality using the Shapiro-Wilk's test. The quotients (16 mg/8 mg; a total of 16 mg /8 mg) of both parameters will be analyzed with ANOVA with dose, period and subject as independent variables. The confidence intervals will be calculated with error taken from the ANOVA.

The correlation between extracted amount of nicotine the pharmacokinetic parameters (C_{max} , T_{max} , AUC_{inf}) will also be investigated.

Efficacy - Visual analogue scale (VAS)

Secondary Objective is to compare each subject's rating of subjective effect of head rush, using a 100 mm visual analogue scale (VAS) anchored with "not at all" to "extremely" at preset time points up to 30 minutes after each test product is administered for:

- craving intensity
- overall "product strength" (head rush, "buzz", "hit", feeling alert)
- increased salivation
- burning sensation in the mouth and/or throat

Determination of sample size

The present study shall assess the comparison of one pharmaceutical NRT preparation to a Swedish snus reference. The sample size calculation is based on the comparison between 6 mg of Nicorette sublingual nicotine tablets, single dose, and SS (8 mg), without loss of

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generality, for simplicity and without adjusting for Type 1 error due to multiple comparisons [Chow, Shao and Wang, 2003].

Linear pharmacokinetics has been shown for buccal administration of nicotine in the dose interval 1-4 mg [Molander L and Lunell E 1999].

A previous single-dose study [Lunell E and Curvall M 2011], showing an average nicotine extraction from the 8 mg SS to be 2.18 ± 0.92 mg made a calculation of sample size possible. Under the assumption of a complete dissolution and extraction of the 6mg of Nicorette sublingual nicotine tablets versus the 2.18 ± 0.92 mg nicotine, extraction leveling off above the 8mg strength for the SS and a standard deviation of 5.0 the estimated sample size is 16 with a power of 80% and $\alpha=0.05$.

The randomization will be performed using Latin Squares approach.

14 ETHICAL REQUIREMENTS

Declaration of Helsinki

The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, and later revisions. The latest version (2008) of the Helsinki Declaration should be included in the protocol as an appendix.

Protocol Reviews

The study will not be started until approval of the protocol, the Subject Information and the Informed Consent Forms have been obtained from the appropriate Ethics Committee/Institutional Review Board of *Lund University, Sweden*. It is the responsibility of the Investigator to forward a copy of the written approval and, where possible, a list of the members, their titles or occupations, and their institutional affiliations, to CROel AB/the Sponsor. The approval should include a study identification and the date of review. The study also requires approval from the Swedish Medical Product Agency. The study will not be started until receipt by CROel AB of written approval from the appropriate authority.

Subject Information and Consent

It is the responsibility of the Investigator to give each subject prior to inclusion in the study, full and adequate verbal and written information regarding the objectives and procedures of the study and the possible risks involved. The subjects must be informed about their right to withdraw from the study at any time. Written subject information (included as an appendix to the protocol) should be given to each subject before enrolment. The written subject information must not be changed without prior discussion with CROel AB/the Sponsor. Furthermore, it is the responsibility of the Investigator to obtain signed Informed Consent Form from all subjects prior to inclusion in the study. The signed Informed Consent Form should be filed by the Investigator for review by the Monitor. The Investigator will confirm receipt of the Informed Consent Form from each subject by signing the appropriate page of the Case Report Form.

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Subject Data Protection

The Investigator should keep a subject identification list not to be available to the Sponsor, including sufficient information to link records, i.e. CRFs and hospital records. The subjects should be informed that the data will be stored and analysed by computer, that Swedish and local regulations for the handling of computerised data will be followed and described in the written subject information and that identification of individual subject data will only be possible for the Investigator. Furthermore, the subjects should be informed about the possibility of inspection of relevant parts of the records by representatives of CROel AB and/or Authorities.

15 FURTHER REQUIREMENTS AND GENERAL INFORMATION

Liability/indemnity/insurance

The Sponsor is liable under law and in accordance with generally accepted standards within the pharmaceutical industry for unexpected injuries, including death, that the use of the study drug may cause subjects.

The Sponsor will indemnify and hold the Investigator as well as any hospital, institution, ethics committee or the alike, harmless from any claims for damages caused by such injuries but only to the extent that the claim is not caused by gross negligence or failure to comply with the protocol and/or governmental regulation by the indemnified.

The Sponsor will require the Investigator to indemnify and hold the Sponsor harmless from any claim caused by gross negligence and/or failure to comply with the protocol and/or governmental regulation by the Investigator.

The Investigator agrees to notify the Sponsor whenever he becomes aware of a claim or action and to co-operate with and authorise the Sponsor to carry out sole management of such claim or action.

The Sponsor's responsibility is covered by product liability insurance. The insurance also covers the Sponsor's liability under law and generally accepted liability standards within industry toward any third parties, including subjects, as Sponsor of the Study. The Investigator's responsibility is covered by liability insurance for scientific studies in human subject with If[®] Insurance Company, Sweden.

Changes to the Final Study Protocol

Any variation in procedure from that specified in the Final Study Protocol may lead to results of the trial being questioned and in some cases rejected. Any proposed protocol change must therefore be discussed with and approved by the Sponsor and submitted for Ethics Committee/Institutional Review Board approval or notification. Any protocol change should be documented in a Protocol Amendment.

Study Time Table

Ethics committee application and MPA application will be submitted in February 2012. Approval is expected in March 2012.

Performance of clinical part of study is expected to start in April 2012 and be finished before the end of June.

Bioanalytical work is scheduled to July-August 2012.

Final Report will be finished in September 2012.

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Discontinuation of the Study

The Sponsor reserves the right to discontinue the study prior to inclusion of the intended number of subjects, but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must call in all participating subjects. At this visit all delivered unused study products and other study materials must be collected and all Case Report Forms be completed as far as possible.

Report and communication of results

All information not previously published concerning the test product and the Sponsor's research, including patent applications, manufacturing processes, basic scientific data, etc., is considered confidential and should remain the sole property of the Sponsor. The Investigator agrees to use this information only in connection with this study and will not use it for other purposes without the written permission from the Sponsor.

After completion of the study, the statistical analysis will be performed by the Investigator. The results will be presented to the Sponsor. Based on these data, CROel AB personnel will prepare a Clinical Study Report. The report may form the basis for a manuscript intended for publication in a medical journal.

It is agreed that the Sponsor has the ownership of all results. Before publication, if publication is agreed upon, the Sponsor will be given the opportunity to review and comment upon the manuscript. The time for review should not exceed 30 days after receipt of the manuscript. If the Investigator has not submitted the results for publication within 6 months after completion of the final Clinical Study Report, the Sponsor will have the right to publish. In this case the Investigator will be given 30 days to review and comment on the manuscript prior to submission to the publisher.

It is agreed between the Investigator and the Sponsor that data from the study will be used by the Sponsor in connection with the development of the study product.

16 REFERENCES

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5. Chow S-C, Shao J and H Wang (2003) Sample Size Calculations in Clinical Research. Marcel Dekker, New York, pp 102-103.
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