STUDY PROTOCOL

Nicotine plasma concentrations and subjective effects of a single dose of General Onyx and General White portion snus compared with 4 mg Nicorette chewing gum

Study Code SM WS 06

Final version

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TABLE OF CONTENTS

1 SUMMARY ............................................................................................................................................. 8
2 BACKGROUND.......................................................................................................................................... 8
3 STUDY DESIGN AND RATIONALE ............................................................................................................. 8
   3.1 Study Design....................................................................................................................................... 8
   3.2 Rationale for Study Design.................................................................................................................. 8
4 TRIAL OBJECTIVES AND PROCEDURE ................................................................................................. 9
   4.1 Primary Objective ............................................................................................................................... 9
   4.2 Secondary Objectives .......................................................................................................................... 9
   4.3 Procedure............................................................................................................................................ 9
5 SELECTION AND WITHDRAWAL OF PATIENTS ....................................................................................... 9
   5.1 Inclusion Criteria................................................................................................................................. 13
   5.2 Exclusion Criteria............................................................................................................................... 14
   5.3 Withdrawal of subjects ...................................................................................................................... 15
6 TREATMENT OF SUBJECTS ................................................................................................................... 11
   6.1 Trial Product....................................................................................................................................... 11
   6.2 Treatment Schedule............................................................................................................................ 11
   6.3 Labeling............................................................................................................................................. 11
   6.4 Prior and Concomitant Therapy ......................................................................................................... 12
   6.5 Treatment Compliance ..................................................................................................................... 12
   6.6 Product Accountability ....................................................................................................................... 12
7 ASSESSMENT OF SAFETY ....................................................................................................................... 17
   7.1 Clinical Safety Assessments ............................................................................................................... 17
   7.1.1 Clinical Interview for Solicitation of Adverse Events ..................................................................... 17
   7.1.2 Vital Signs..................................................................................................................................... 17
7.2 Efficacy Assessments ........................................................................................................... 18
7.3 Laboratory Safety Assessments .......................................................................................... 18
7.4 Pharmacokinetic Assessments ............................................................................................ 18
  7.4.1 Sampling Procedures and analysis of used gum .............................................................. 18
  7.4.2 Drug Concentration Measurements in plasma .............................................................. 18
  7.4.3 Pharmacokinetic Calculations ....................................................................................... 19
7.5 Adverse Events ................................................................................................................... 20
  7.5.1 Description .................................................................................................................... 20
  7.5.2 Follow-Up of Adverse Events ....................................................................................... 23
8  STATISTICS .......................................................................................................................... 24
  8.1 Sample Size and randomization ....................................................................................... 24
  8.2 Pharmacokinetics .............................................................................................................. 24
9  QUALITY CONTROL (QC) AND QUALITY ASSURANCE (QA) ........................................... 24
10 STOPPING RULES / DISCONTINUATION CRITERIA ......................................................... 25
11 ETHICS .................................................................................................................................. 25
  11.1 Ethical Conduct of the Trial ............................................................................................. 25
  11.2 Independent Ethics Committee (IEC) ............................................................................. 25
  11.3 Subject Information and Consent .................................................................................... 25
12 DATA HANDLING AND RECORD KEEPING ..................................................................... 26
  12.1 Case Report Forms .......................................................................................................... 26
  12.2 Record Retention ............................................................................................................. 26
13 COMPARISON TO NICORETTE GUM ............................................................................... 25

APPENDICES
Appendix 1  DECLARATION OF HELSINKI
Appendix 2  SCHEDULE OF ACTIVITIES
Appendix 3  INFORMED CONSENT
SYNOPSIS

Study code: SM WS 06

Title: Nicotine plasma concentrations and subjective effects of a single dose of General Onyx and General White portion snus compared with 4mg Nicorette chewing gum.

Primary Objective: To compare each subject’s plasma concentration of nicotine at 30 minutes ($C_{30}$) after administration of one single dose of General Onyx portion snus to that of one piece of Nicorette 4 mg chewing gum.

Secondary Objective: From the nicotine plasma concentration-time curves the $T_{max}$, $C_{max}$, $AUC_{inf}$, $T_{1/2}$ and $V_d$ will be calculated. Secondary objective is to compare these variables, including General White 1g portion snus to the Nicorette 4 mg chewing gum. To compare each subject’s rating of subjective effects using a 100 mm visual analogue scale (VAS) anchored with "not at all" to "extremely" at the time points of blood sampling.

Total sample size: The study will include 15 subjects. Linear pharmacokinetics has been shown for buccal administration of nicotine in the dose interval 1-4 mg. From a pre-study a plasma nicotine concentration at 30 minutes of about 6 ng/ml for the 4 mg Nicorette® 4 mg chewing gum may be expected and about 13 ng/ml for the General Onyx snus. To detect a difference between the General Onyx snus, and the 4 mg Nicorette® gum of 6 ng/ml +/- 4.9 with a power of 80% and alpha=0.05 twelve subjects will be needed.

Study design: Open, three-way cross-over. Single dose administration. The treatment sequence is randomized. The two General brands of snus and Nicorette 4 mg chewing gum are tested. Subjects report to the laboratory for three experimental sessions. After baseline measurements, plasma nicotine concentrations are monitored over 8 hours. Each subject’s rating of subjective symptoms, e.g. product "strength" using a 100 mm visual analogue scale (VAS) anchored with "not at all" to "extremely" is recorded up to 30 minutes after dosing.

Subject population 18-50 years old, male and female, healthy volunteer, smoking
≥7 cigarettes per day. No use of smokeless tobacco and nicotine containing products is allowed. Subjects shall be fasting overnight.

**Test articles:**
General Onyx 1 g portion snus. The subject keeps one pouch of snus still between the upper lip and the gum for 30 minutes.
Batch No:
General White 1 g portion snus.
Batch No:

**Reference article:**
Nicorette 4 mg chewing gum. Nicorette is chewed according to instructions in package insert.
Batch No:

**Procedure:**
The treatments are given as single doses in randomized order. Serial blood samples are drawn before, and at regular time intervals up to 8 hours after administration (14 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 area is based on plasma data corrected for background nicotine (time zero sample).

*Subjective effects* — Each subject’s rating of product "strength" using a 100 mm visual analogue scale (VAS) anchored with "not at all" to "extremely". Serial VAS scores will be obtained before and at regular time intervals after the test and reference products are administered:

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

**Analysis:**
Assessment of the difference between the General Onyx snus, and the 4 mg Nicorette® gum in plasma concentration of nicotine at 30 minutes (C_{30}). Difference between T_{max}, C_{max}, and AUC_{inf} for General Onyx vs Nicorette 4 mg and General Onyx vs Nicorette 4mg. Differences between VAS scores of subjective effects for General Onyx portion snus vs Nicorette 4 mg and General White portion snus vs Nicorette 4mg.

**Study site:**
CROEL AB
Specialisthuset i Eslöv AB,
Eslöv, SWEDEN
SUMMARY

Fifteen healthy smokers are given single doses of General Onyx 1 g portion snus and General White 1g portion snus, respectively, and one piece of Nicorette 4 mg chewing gum. Serial blood samples are drawn before (0), 2, 4, 8, 16, 24, 30, 45, 60 minutes, 1.5, 2, 4, 6 and 8 hours after administration for determination of nicotine. Mean ± SD extracted dose of nicotine from each preparation will be estimated. The percentage extracted of the nicotine content will be calculated. $C_{max}$ and $T_{max}$ will be estimated. A comparison to Nicorette 4 mg chewing gum will be made. The dose absorbed into the systemic blood circulation (AUC) in relation to the dose following the 4 mg Nicorette chewing gum will be calculated. Self-reports of subjective effects will be obtained up to 30 minutes after the test product is administered using a 100 mm visual analogue scale (VAS) anchored with "not at all" to "extremely".

The subjects are male and female smokers, smoking a minimum of 7 cigarettes per day. They should have no history of cardiac, kidney or hepatic disease, alcohol abuse or drug dependence. A physical examination including ECG and blood pressure should give no evidence of disease. No abnormalities should be found in a routine laboratory screening.

The subjects are requested not to smoke or to use any other form of nicotine containing products from 8 p.m. the day before each session until the last blood sample in each session. Previous experience has shown that subjects that have abstained from smoking for 12 hours have a plasma nicotine value of $\leq 4$ ng/ml. A value >4 ng/ml prior to start of administration should lead to exclusion from statistical analysis. The subjects must be fasting overnight from 12 p.m. the day before each session. No food and drink are allowed from 15 minutes prior to and until 60 minutes after drug administration.

2. 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. Snus products are capable of rapidly delivering nicotine to the bloodstream, and therefore may be more satisfactory than e.g. NRT products.
3. STUDY DESIGN AND RATIONALE

3.1 Study design

The study has an open, three-way cross-over, design. The preparations are given as single oral administrations on three separate occasions. The sequence of treatments is randomized. Serial blood samples are drawn for determination of nicotine levels before, during and after drug administration.

3.2 Rationale for Study

The pharmacokinetics of four brands of US moist snuff has been explored (1). However, Swedish snus pharmacokinetics is not well investigated (2) and a comparison to high dose (4mg) Nicorette gum has not been published. Snus availability in Sweden is claimed to have contributed to the unusually low rates of smoking among Swedish men by helping them transfer to a less harmful form of nicotine dependence (3). Swedish snus typically has a pH in the range 7.8-8.5 with only minor differences between brands (4) and therefore probably gives an efficient delivery of nicotine.

Comparisons of health consequences between cigarettes and smokeless tobacco (ST) show that cigarette smoking produces more negative health effects, is likely to have a higher addiction potential and more severe withdrawal, and leads to a higher rate of relapse than ST use, as reported in a review by Hatsukami et al (5). Smokers who are highly dependent on nicotine have particular problems stopping smoking and very low 12-month success rates with all NRT. Smokeless tobacco (ST) substitution for cigarettes as a smoking cessation method or as a means to reduce cigarettes has been proposed. The impact of using ST in these ways is relatively unknown to date. Hatsukami et al concluded that considerably more research and product regulation is necessary prior to considering smokeless tobacco as a harm reduction method.

A pilot study has shown faster absorption of nicotine from portion snus than from nicotine chewing gum. A comparison of the characteristics of the blood nicotine curves between the high dose (4mg) Nicorette and snus therefore seems warranted. Swedish snus is also an alternative to the Nicorette 4 mg chewing gum for buccal nicotine administration to smokers who cannot use the gum because of problems with dentures or aerophagia.
4. TRIAL OBJECTIVES AND PROCEDURE

4.1 Primary Objective
Plasma samples drawn at regular intervals for up to 8 hours after each dose administration will be analyzed for nicotine. The primary objective of the present study is to compare each subject’s nicotine plasma concentration ($C_{30}$) of nicotine at 30 minutes after administration of one single portion of General Onyx 1 g snus and one piece of Nicorette 4 mg chewing gum, respectively.

4.2 Secondary Objectives
From the nicotine plasma concentration-time curves the $T_{max}$, $C_{max}$, $AUC_{inf}$, $T_{1/2}$ and $V_d$ will be calculated. A Secondary Objective is to compare these variables. A Secondary Objective is also to compare each subject’s rating of subjective effects using a 100 mm visual analogue scale (VAS) anchored with "not at all" to "extremely" at preset time points up to 30 minutes after the test product is administered. One more Secondary Objective is also to compare and General White 1g snus to Nicorette 4 mg chewing gum.

4.3 Procedure
Study site and timetable
The study is performed at Specialisthuset i Eslöv AB, Eslöv, Sweden, in spring-summer 2006. This is a Phase I, open, single-center trial in healthy male and female smokers. Each subject’s participation will be approximately one month during which the investigational drugs will be administered on three occasions. Time interval between sessions will be at least 6 days.

Visit 0 - Screening visit:
At this visit eligibility to participate in the study will be checked. The subject will be given information about the study procedures and must sign the informed consent form. At this visit the following procedures will be conducted and documented:

- Informed consent
• Inclusion/Exclusion criteria
• Demographic data
• Body height and weight
• Supine blood pressure and heart rate
• Medical history
• Concurrent diseases/symptoms
• Concomitant medication
• ECG
• Physical examination
• Urinalysis
• Blood samples drawn for clinical chemistry, hematology and virology (HIV, hepatitis)

If the subject fulfills all criteria for enrolment appointments for visits 1-3 will be booked.

Visits 1-3
At every visit the following procedures will be conducted and documented:

**Predose:**

• Confirmation of eligibility - Exhaled Carbon Monoxide

To confirm that each subject has abstained from smoking a test for exhaled carbon monoxide (ECO) is performed at each visit. Levels of ECO up to 11 ppm are considered compatible with abstinence[^3].

• Concomitant medication
• An intravenous cannula will be administered for the blood sampling
• Blood sample (5 ml) for PK analysis (baseline=0 minutes).

*Administration of investigational drug:* General Onyx 1 g and General White 1g snus and Nicorette 4 mg chewing gum, respectively (= 0 minutes).
Blood sampling

Venous blood samples (5 ml) are collected in sodium heparinised Venoject glass tubes from an antecubital vein at the following time-points:

before (0), 2, 4, 8, 16, 24, 30, 45, 60 minutes, 1.5, 2, 4, 6 and 8 hours after administration.

Only non-smoking personnel are allowed to perform practical functions in this study. Drinking coffee and carbonated beverages is not permitted while using the study preparations, since they block absorption of nicotine from nicotine polacrilex gum (5). The blood samples are cooled and centrifuged within 30 minutes at 1000g for 10 minutes at ambient room temperature. The plasma is then separated and transferred to cryotubes which are frozen within 30 minutes and kept frozen (-20°C) pending analysis. The plasma samples are shipped by courier door to door on ice to ABS Laboratories, London, England.

5. SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria

1. Consent to participate voluntarily and sign Informed Consent Form prior to any study procedure.
2. Healthy male and female, age 18 through 50 years.
3. Willing and able to take oral medication.
4. Willing and able to comply with the study specific procedures.
5. Smoker of ≥7 cigarettes/day.
6. Fasting overnight from 12.00 p.m.
5.2 Exclusion Criteria

Subjects fitting any of the following characterizations are excluded from the study:

1) Use of snuff.

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 male and females or > 470 msec for female and females); other clinically significant heart conditions which would negatively impact on the patient completing the study.

3) Subjects with clinically significant liver disease which may prevent the patient 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 patient 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 patient’s participation in the clinical study.

6) Subjects who are participating in other drug studies or who have received other investigational drugs within 30 days prior to enrolment. Additionally, subjects previously included into this study and whom then dropout of the study is not to be re-entered into the study.

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 (e.g., barbiturates, rifampin, carbamazepine, phenytoin, primidone) 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.

5.3 Withdrawal of subjects

A subject should be withdrawn from the trial treatment if, in the opinion of the investigator, it is medically necessary, or if it is the wish of the subject. In any circumstance, subject outcome should be documented and a Study Termination Report completed.

The subjects must abstain from nicotine for at least 12 hours prior to administration of the General Onyx 1 g and General White 1g snus and the Nicorette 4 mg chewing gum, respectively, and during the whole of the experimental session. Previous experience has shown that subjects that have abstained from smoking for 12 hours have a plasma nicotine value of <4 ng/ml.

6. TREATMENT OF SUBJECTS

6.1 Trial Products

Three treatments are given as single doses in randomized order on separate occasions.

Treatment A: General Onyx 1 g portion snus.
Batch No:

Treatment B: General White 1g portion snus.
Batch No:

Treatment C: Nicorette 4 mg chewing gum.
Batch No:

6.2 Treatment Schedule

The preparations are used over 30 minutes. The subject keeps the pouch of snus between the upper lip and the gum for 30 minutes. The gum is chewed (One chew/2 seconds. Metronome is used) over 30 minutes. Saliva must be swallowed once every minute.
The subjects are not allowed to eat or drink coffee and carbonated beverages or any other liquid for the first 60 minutes after dose administration [6].

6.3 Labeling

General Onyx 1 g and General White 1g snus, containing approximately 11mg nicotine and 8mg, respectively, releasing approximately 2.7mg and 3mg, will be delivered in its original pack from Swedish Match AB, Stockholm, Sweden. Nicorette® chewing gum, containing 4 mg nicotine and releasing approximately 3 mg, will be delivered in its original blister pack as available in the open market in Sweden from the pharmacy. Labeling will be in Swedish. Individual packaging will be made at the Hospital Pharmacy, Helsingborg, by the responsible pharmacist.

The package will be labelled:
General Onyx 1 g and General White 1g snus. Nicotine 4 mg chewing gum.

For clinical trial.

CTN: SM WS 06
Subject No.: 1 (15)
Treatment.: A (B,C)
Batch No.: 

Expiry date:
Dosage: according to physician’s instruction
Responsible investigator: Erik Lunell, M.D.
Croel AB, Helsingborg, Sweden.
Keep out of reach of children.

6.4 Prior and concomitant therapy

Oral contraceptives are allowed. OTC drugs are allowed up till 24 hours before and after each dose of study medication. No prescription drugs or herbal remedies are allowed. Any such use in between study days should be reported to the investigator. Concomitant prescription drugs during the study considered necessary for the subject's welfare are allowed at the discretion of the investigator. All concomitant medication will be recorded in appropriate section in the CRF. No other drug under investigation may be used concomitantly with the study drug. The subjects are not allowed to participate concurrently in any other study.
6.5 Treatment compliance
Each dose will be taken under supervision of the staff at the trial site. To confirm that each subject has abstained from smoking a test for exhaled carbon monoxide (ECO) is performed at each visit. Levels of ECO up to 11 ppm are considered compatible with abstinence [3].

6.6 Product accountability
All trial medication must be accounted for and any discrepancies explained. The investigator or pharmacy is responsible for keeping detailed records. A product accountability form is available in the CRF to document the dispensed medication for each subject.

Trial medication will be released from the Hospital Pharmacy of Helsingborg to the Investigator when all necessary approvals have been granted. Date, amount subject numbers and investigator or pharmacist’s signature will be recorded on the trial product inventory. At the end of the trial, any unused trial medication must be returned to CROel AB according to standard hospital procedure. Destruction of returned product and its documentation is the responsibility of CROel AB.

7 ASSESSMENT OF SAFETY

7.1 Clinical Safety Assessments

7.1.1 Clinical Interview for Solicitation of Adverse Events
A clinical interview for solicitation of adverse events will be performed at the time points given in the Schedule of Activities.

7.1.2 Vital Signs
Supine heart rate will be measured at the time points 0, 10, 20 and 30 minutes (Appendix 2 SCHEDULE OF ACTIVITIES).
7.2 **Efficacy Assessments**

*Subjective effects* — Each subject’s rating of product "strength" using a 100 mm visual analogue scale (VAS) anchored with "not at all" to "extremely". VAS scores obtained at the time points 0, 10, 20 and 30 minutes after snuff is administered:

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

7.3 **Laboratory Safety Assessments**

All clinically significant abnormal findings should be reported as Adverse Events as defined in section 8.

7.4 **Pharmacokinetic Assessments**

7.4.1 **Sampling Procedures and analysis of used snus and gum**

The nicotine content of unused General Onyx 1 g and General White 1g snus, respectively, per portion will be estimated. The mean ± SD extracted dose of nicotine from one General Onyx 1 g and General White 1g portion of snus, respectively, 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. The Nicorette 4 mg gum will be assayed using the same procedure.

7.4.2 **Drug Concentration Measurements in plasma**

Frozen plasma samples collected for nicotine level determinations will be shipped to a certified contract laboratory, ABS Laboratories, London, England, for analysis. Collection and shipping will be handled according to procedures listed in Appendix 4. The determination
of nicotine will be performed using capillary gas chromatography (GC-MS) after a single liquid-liquid extraction of a basified plasma sample (7).

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 ±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 quantitation is 0.6 ng/ml.

### 7.4.3 Pharmacokinetic Calculations

Plasma samples drawn at regular intervals for up to 8 hours after each dose administration will be analyzed for nicotine. Pharmacokinetic parameters will be calculated based upon plasma concentration - time data by model-independent methods, using the WinNonlin Professional® computer system for pharmacokinetic data analysis (Pharsight Corporation, Mountain View, CA 94040, USA). The pharmacokinetic parameters \( \text{AUC}_{\text{inf}}, \text{C}_{\text{max}}, \text{T}_{\text{max}}, \text{T}_{1/2} \) and \( \text{V}_d \) will be the primary variables.

**AUC\text{inf}:** the total area under the plasma concentration versus time curve, calculated by the log/linear trapezoidal method from time zero to the last quantifiable concentration (\( \text{C}_{\text{last}} \)) and by extrapolation to infinity by \( \text{C}_{\text{last}}/\lambda_\text{e} \), where \( \lambda_\text{e} \) is the elimination rate constant estimated from individual linear regression on the terminal part of the log concentration versus time curve.

**AUC\text{t}:** the area under the plasma concentration versus time curve from time zero to the last quantifiable concentration (\( \text{C}_{\text{last}} \)), calculated by the log/linear trapezoidal method.

**C\text{max}:** the observed maximum plasma concentration.

**T\text{max}:** the time required to reach \( \text{C}_{\text{max}} \).

**T\text{1/2}:** the plasma elimination half-life, calculated by \( \ln(2)/\lambda_\text{e} \).
7.5 Adverse Events

7.5.1 Description

Definition

An adverse event (AE) is any untoward medical occurrence in a patient or trial subject administered a drug or biologic (medicinal product) or using a medical device; the event does not necessarily have a causal relationship with that treatment or usage. Adverse events include the following:

a. All suspected adverse reactions.

b. All reactions from medication overdose, abuse, withdrawal, sensitivity, or toxicity.

c. Apparently unrelated illnesses, including the worsening of a preexisting illness (see Preexisting Conditions, below).

d. Injury or accidents. Note that if a medical condition is known to have caused the injury or accident (e.g., a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) should be reported as two separate adverse events. The outcome of the accident (e.g., hip fracture secondary to the fall) should be recorded under Comments.

e. Abnormalities in physiological testing or physical examination (findings that require clinical intervention or further investigation beyond ordering a repeat [confirmatory] test).

f. Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event. Laboratory abnormalities associated with a clinical event (e.g., elevated liver enzymes in a patient with jaundice) should be described under Comments on the report of the clinical event rather than listed as a separate adverse event.

Preexisting Conditions

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

Procedures

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

**Adverse Event Reporting Period**

The adverse event-reporting period for this trial begins upon receiving the first dose of investigational and ends at the final clinic visit. All adverse events that occur in trial patients during the adverse event reporting period specified in the protocol must be reported to Swedish Match AB, WHETHER OR NOT THE EVENT IS CONSIDERED MEDICATION/PRODUCT RELATED.

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

**Seriousness (Gravity)**

Each adverse event is to be classified by the investigator as **SERIOUS** or **NONSERIOUS**. This classification of the gravity of the event determines the reporting procedures to be followed.

An adverse event that meets one or more of the following criteria/outcomes is classified as serious:

- Death
- Life-threatening (i.e., immediate risk of death)
- In-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect

**Other, Medical/Scientific Judgment**

Medical judgment should be exercised in deciding whether a reaction is serious in other situations. Important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient should be considered serious.
Eliciting Adverse Event Information

The investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the trial subject. In addition, each trial subject will be questioned about adverse events for each dose as listed in appendix 2. The question asked will be “Have you noticed any changes in your health since we asked last?”

Reporting

If a SERIOUS adverse event occurs, the Swedish Match AB monitor is to be notified using the SERIOUS ADVERSE EVENT REPORT (SAER) form (or within 24 hours of awareness of the event by the investigator). The initial report is to be followed by submission of more detailed adverse event information on the SAER form within 5 working days of the event. If unexpected, serious adverse events are also to be reported immediately to the Independent Ethics Committee and to the Swedish Medical Products agency. Please review the table below.

Serious adverse events should also be reported on the clinical trial adverse event case report form.

Note: The SAER form is not the same as the adverse event case report form, however, where the same data is collected, the forms must be completed in a consistent manner. For example, the same adverse event term should be used on both forms.

NON-SERIOUS adverse events are to be reported on the adverse event case report forms, which are to be submitted to Swedish Match AB as specified in the adverse event report submission procedure for this protocol.

REPORTING REQUIREMENTS FOR ADVERSE EVENTS

<table>
<thead>
<tr>
<th>Gravity</th>
<th>Reporting Time</th>
<th>Type of Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERIOUS</td>
<td>Within 24 hours</td>
<td>Initial report on SAER</td>
</tr>
<tr>
<td></td>
<td>Within 5 working days</td>
<td>Final report on SAER</td>
</tr>
<tr>
<td>NONSERIOUS</td>
<td>Per case report form</td>
<td>Appropriate case report</td>
</tr>
<tr>
<td></td>
<td>submission procedure</td>
<td>forms</td>
</tr>
</tbody>
</table>

NOTE: In the rare event that the investigator does not become aware of the occurrence of a serious adverse event immediately (for example, if an outpatient trial subject initially seeks treatment elsewhere), the investigator is to report the event within
24 hours after learning of it and document his/her first awareness of the adverse event.

**Recording Instructions**

Adverse events are to be recorded in the case report forms as specified.

If required on the adverse event case report forms, the investigator will use the adjectives MILD, MODERATE, or SEVERE to describe the maximum intensity of the adverse event.

For purposes of consistency, these intensity grades are defined as follows:

- **MILD**
  Does not interfere with subject's usual function

- **MODERATE**
  Interferes to some extent with subject's usual function

- **SEVERE**
  Interferes significantly with subject's usual function

Note the distinction between the gravity and the intensity of an adverse event. **Severe** is a measure of intensity; thus, a severe reaction is not necessarily a **serious** reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria for serious events listed above.

The investigator will also be asked to assess the possible relationship between the adverse event and the investigational medication as well as any concomitant medications.

**7.5.2 Follow-Up of Adverse Events**

All adverse events should be followed until they are resolved or the subject’s participation in the trial ends. Instructions for reporting changes in an ongoing adverse event during a subject's participation in the trial are provided in the instructions that accompany the adverse event case report forms.

In addition, all serious adverse events 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 resolve or until the investigator assesses them as “chronic” or “stable.” Resolution of such events is to be documented on the appropriate CRF.
8 Statistics

8.1 Sample size and randomization

Linear pharmacokinetics has been shown for buccal administration of nicotine in the dose interval 1-4 mg. From a pre-study a plasma nicotine concentration at 30 minutes of about 6 ng/ml for the 4 mg Nicorette® 4 mg chewing gum may be expected and about 13 for the General Onyx 1 g snus. To detect a difference between the General Onyx 1 g snus, and the 4 mg Nicorette® gum of 6 ng/ml +/- 4.9 with a power of 80% and alpha=0.05 twelve subjects will be needed. The study will include 15 subjects. Drop-outs will be replaced. The randomization will be performed using Latin Squares approach.

8.2 Pharmacokinetics

Plasma samples drawn at regular intervals for up to 8 hours after dose administration will be analyzed for nicotine. From these curves the T_max, C_max, AUC_infinity, T_1/2 and V_d will be calculated.

The comparison between the General Onyx 1 g and General White 1g snus, respectively, and the 4 mg Nicorette® regarding the T_max, C_max, AUC_infinity, T_1/2 and V_d will be analysed using Wilcoxon Rank Sum test.

The calculation will be based on a standard error estimated from a three period crossover analysis of variance model accounting for the following sources of variation: Sequence, subject (sequence), period, treatment and carry-over (only included in model if found significant).

9 Quality control (QC) and quality Assurance

Monitoring visits to the trial site will be made periodically 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. The trial site may also be subject to quality assurance (QA) audit by an external auditor appointed by CROel AB as well as inspection by appropriate regulatory agencies. The investigator/institution guarantee access to source documents by the CROel monitor, the quality assurance auditor and appropriate regulatory
It is important that the investigator and their relevant personnel are available during the monitoring visits and possible audits and that sufficient time is devoted to the process.

10 Stopping Rules / Discontinuation Criteria
CROel AB reserves the right to discontinue the trial 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 contact all participating subjects within one week.

11 Ethics

11.1 Ethical Conduct of the Trial
The trial 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 as well as ICH and GCP guidelines.

11.2 Independent Ethics Committee (IEC)
It is the responsibility of the investigator to obtain approval of the trial protocol/amendments from the IEC of the University of Lund, Sweden. The investigator should file all correspondence with the IEC. Copies of IEC approvals should be forwarded to CROel AB.

11.3 Subject Information and Consent
It is the responsibility of the investigator to give each subject prior to inclusion in the trial, full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. The patients must be informed about their right to withdraw from the trial at any time. Written subject information (a proposed informed consent is attached as appendix 6) must be given to each subject before enrolment. Furthermore, it is the responsibility of the investigator to obtain signed informed consent from all subjects prior to inclusion in the trial.
12  Data handling and record keeping

12.1  Case Report Forms
A Case Report Form (CRF) is required and should be completed for each included subject. The completed original CRFs are the sole property of Swedish Match AB and should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from Swedish Match AB.

12.2  Record Retention
To enable evaluations and/or audits from Health Authorities/Swedish Match AB, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records, e.g., CRFs and hospital records), all original signed Informed Consent Forms, copies of all CRFs and detailed records of drug disposition. To comply with international regulations, the investigator should retain the records for 15 years.

13  COMPARISON TO NICORETTE 4 mg CHEWING GUM

The primary objective is to assess the plasma nicotine levels achieved at 30 minutes following administration of a single dose of General Onyx 1 g and General White 1g portion snus, respectively. The resulting plasma concentration-time curves will be presented separately for each individual and the corresponding data will be tabulated. 

$C_{\text{max}}$ and $t_{\text{max}}$ after the snus and gum will be tabulated. The corresponding $C_{\text{max}}$ and $t_{\text{max}}$ after the administration of Nicorette 4 mg chewing gum will be reported. Comparison of the nicotine plasma concentration at 30 minutes after dose administration will be made. The AUC of the General Onyx 1 g and General White 1g snus, respectively, and the corresponding value following use of the reference Nicorette 4 mg gum will be used for comparison of the relative bioavailability of portion snus, i.e. the dose absorbed into the systemic blood circulation as percent of that of the 4 mg Nicorette chewing gum will be calculated. The extracted dose from the 4 mg Nicorette gum is approximately 70%, i.e. about 3 mg, and the bioavailability of the extracted dose is approximately 60%, i.e. about 1.8 mg.
14 REFERENCES


