CLINICAL PHARMACOLOGY STUDY REPORT	
STUDY PRODUCTS	"General" 1 g portion
	"Catch" 1 g portion
	"Catch Mini" 0.5 g portion
	"Catch Dry Mini" 0.3 g portion
	Nicorette Chewing 2 mg gum
VERSION NO.	3 (FINAL)
STUDY CODE	**SM WS02**
DATE	2003-09-22

An open-label cross-over study of nicotine plasma levels achieved following repeated use of four different types of snus and Nicorette chewing gum.

Investigators and study administrative structure

Principal Investigator and pharmacokineticist

Erik Lunell, M.D., Ph.D.

CROEL HB
Nils Bjelkegatan 2B
SE-222 20 Lund, Sweden
Telephone: +46 46-12 04 60
Telefax: +42 42-913 92
e-mail: croel @swipnet. se

Signature Date

Sponsor's Representative

Margareta Curvall Ph. D.
Forskning och Analys
Swedish Match Northern Europe
Rosenlundsgatan 36
SE-118 85 Stockholm, Sweden
Telephone: +46 8 658 04 44
Telefax: +46 8 668 97 77
e-mail: margareta.curvall@swedishmatch.se

Signature Date

Statistician
Fredrik Hansson
HH-Statistik
Planteringsv. 11, SE-244 65 Furulund, Sweden

Telephone: +46 46 73 82 87
Telefax: +46 46 73 82 73

Responsible bioanalyst
Colin Feyerabend
ABS Laboratories
Wardalls Grove, Avonley Road
London SE14 5ER, England

Telephone: +44 207 277 5572
Telefax: +44 207 277 5572

Monitor
Marianne Lunell
Reg. Pharmacist
CROEL HB
Nils Bjelkegatan 2B
SE-222 20 Lund
Sweden

Telephone: 046-12 04 60
Telefax: 042-913 92

Responsible analysts
Christina Andersson
Ann-Marie Eklund
Eva Norlén Moritz
Swedish Match Northern Europe
Rosenlundsgatan 36
SE-118 85 Stockholm, Sweden

Telephone: +46 8 658 02 00
Telefax: +46 8 668 97 77

STUDY PERIOD

- **FIRST SUBJECT ENROLLED:** 7 November 2002
- **LAST SUBJECT COMPLETED:** 7 December 2002

The study was conducted in accordance with the principles of Good Clinical Practice.

TABLE OF CONTENTS

SIGNATURE PAGE……2
SYNOPSIS ……5
1 ETHICS ……8
2 INTRODUCTION……8
3 STUDY OBJECTIVES……10
4 OVERALL STUDY DESIGN……10
5 STUDY SITE AND TIME TABLE……10
6 MATERIAL AND METHODS……11
6.1 Subjects……11
6.2 Study Products……12
6.3 Treatment regimens……13
6.4 Concomitant Medication and Treatment Compliance……14
6.5 Blood Sampling……14
6.6 Bioanalysis of Nicotine in Plasma……15
6.7 Residual Nicotine in used snus……15
6.8 Residual Sodium Chloride in used snus……15
6.9 Pharmacokinetic Calculations……15
7 ASSESSMENT OF SAFETY……16
8 DATA MANAGEMENT AND STATISTICS……19
8.1 Randomization……19
8.2 Data management……19
8.3 Statistical considerations……19
9 QUALITY CONTROL (QC) AND QUALITY ASSURANCE (QA)……19
10 RESULTS……20
10.1 Demographics……20
10.2 Nicotine Extraction from Snus and gum……20
10.3 Sodium Chloride Extraction from Snus……19
10.4 Maximal Nicotine Concentration……22
10.5 Time to Maximal Nicotine Concentration……24
10.6 Area Under the Plasma concentration - Time Curve (AUC)……25
10.7 Cmax Ratio of Snus / Nicorette gum……26
10.8 AUC Ratio of Snus / Nicorette gum……26

11 RELATIVE BIOAVAILABILITY 27

12 ADVERSE EVENTS 27

13 DISCUSSION AND CONCLUSIONS 28

REFERENCES.....29

APPENDICES

Appendix 1 Individual data on nicotine extraction.

Appendix 2 Individual plasma nicotine concentrations. Tabulated.

Appendix 3 Individual plasma nicotine concentrations. “Spagetti plots”.

Appendix 4 Data on sodium chloride extraction.

SYNOPSIS

Study code: SM WS02

Title: An open-label cross-over study of nicotine plasma levels achieved following repeated use of four different types of snus and Nicorette chewing gum.

Primary objective: To estimate nicotine plasma levels achieved following one day's use of four different types of snus compared with 2 mg Nicorette chewing gum.

Total sample size:

No. planned	12
No. randomised and treated	12
No. analysed for pharmacokinetics	11 (10 complete)
No. analysed for safety	12
No. completed	12

Study design: Single centre, open label, randomised, cross-over, repeated dose study.

Included subjects: 18-23 (19.8±1.3) years old, male non-smoking healthy volunteers, regularly using 4 - 17 portions snus daily since minimum 1 year.

Test products:

A. "General" 1 g portion snus containing 8.8±0.4 mg nicotine per portion.

B. "Catch" Licorice 1 g portion snus containing 7.0±0.1 mg nicotine per portion.

C. "Catch Mini" 0.5 g portion snus containing 4.5±0.3 mg nicotine per portion.

D. "Catch Dry Mini" 0.3 g portion snus containing 4.8±0.6 mg nicotine per portion.

Comparator product:

E. Nicotine gum (Nicorette®) containing 1.9±0.1 mg nicotine.

Dosage and mode of administration: The General, Catch Licorice, Catch Mini and Catch Dry Mini snus portions were administered once every hour for 11 hours (12 administrations) and were kept between the upper lip and the gum for 30 minutes. The 2 mg Nicorette® chewing gum was administered every hour for 11 hours (12 administrations) and was chewed over 30 minutes at each time.

Procedure:

Serial venous blood samples, a total of 325 ml, were drawn for determination of nicotine levels at the following time-points: before (0) and 1, 3, 5, 7, 9 hours after the firs dose administration. Blood sampling took place just before each dosing (pre-dose). Following the last dose at 11, 11+10', 11+20', 11+30', 11+40', 11+50' and 12 hours after the first dose administration.

Main measurements and variables:

Pharmacokinetic: Extracted dose of nicotine. Plasma levels of nicotine once hourly (pre-dose) and during the hour following the last dose administration. Area under the plasma concentration-time curve (AUC) and maximum plasma concentration (C_{max}) of the last dosing interval. AUC reflects the size of the dose bioavailable, i.e. absorbed into the systemic blood circulation.
Pharmacodynamic: Not applicable in this study.
Safety: Adverse Events (AE).

Analysis:

Descriptive statistics were used to summarise data. Individual and mean plasma curves of each type of snus. Frequency distributions of C_{max} and T_{max} for the last dose. Approximate relative bioavailable dose of each type of snus, calculated using AUC of Nicorette® 2 mg gum as reference (extracted dose 0.84 mg, bioavailable dose 0.46 mg).

Pharmacokinetic results:

The mean nicotine amount extracted, was 2.74±0.80, 1.55+0.68, 2.00+0.56 and 1.08+0.94 mg/portion for General, Catch Licorice, Catch Mini and Catch Dry Mini snus, respectively.
The mean C_{max} obtained in the last dosing interval after use of the snus was 29.0 ± 8.5, 23.8 ± 8.6, 21.0 ± 6.9 and 10.9 ± 5.7 ng/ml for General, Catch Licorice, Catch Mini and Catch Dry Mini" snus, respectively. The mean C_{max} obtained after chewing of the 2 mg Nicorette® gum was 12.8 ± 4.7 ng/ml. The median t_{max} was 30 minutes for all products.
The mean C_{max} ratio versus Nicorette® 2 mg chewing gum was 2.5 ± 1.0, 2.0 ± 0.7, 1.8 ± 0.6 and 0.9 ± 0.5 for General, Catch Licorice, Catch Mini and Catch Dry Mini snus, respectively.
The mean AUC of the last dosing interval was 26.2 ± 3.4, 21.6 ± 8.8, 19.0 ± 6.7 and 9.8 ± 5.1 h ∗ ng/mL for General, Catch Licorice, Catch Mini and Catch Dry Mini snus, respectively. The mean AUC after chewing of the 2 mg nicotine polacrilex gum (Nicorette®) was 11.6 ± 4.5 h ∗ ng/mL.
The mean AUC ratio versus Nicorette® 2 mg chewing gum was 2.6 ± 1.0, 2.0 ± 0.8, 1.8 ± 0.6 and 0.9 ± 0.5 for General, Catch Licorice, Catch Mini and Catch Dry Mini snus, respectively.

Safety results:

All snus brands were very well tolerated and accepted. Adverse events were reported approximately 2-3 times per session for the Nicorette® chewing gum. Most frequently reported were hiccups,

headache and irritated throat, with occasional reports of abdominal discomfort, cough and nausea. For the various brands of snus as well as for the Nicorette® chewing gum craving and withdrawal were reported in the morning hours of each session. With respect to craving and withdrawal General was most liked and Catch Dry Mini was least liked.

Summary:

Catch Dry Mini was close to bioequivalent to the Nicorette® 2 mg chewing gum. Catch Licorice 1g and Catch Mini 0.5 g were quite similar, with AUC and C_{max} twice those for Nicorette® 2 mg gum. The AUC and C_{max} of General were almost 2.5 times those for Nicorette® 2 mg gum. Compared to smoking Catch Dry Mini once hourly produced blood levels similar to the lower end (7-10 cigarettes/day) of cigarette smoking, while Catch Licorice and Catch Mini once hourly showed blood levels similar to moderate cigarette smoking (15-20 cigarettes/day). General once hourly produced blood levels similar to the upper end (25-40 cigarettes/day) of cigarette smoking.
The mean sodium chloride amount extracted, was 8.13±7.33, 10.38±6.83, 5.58±4.49 and 4.73±6.61 mg/portion for General, Catch Licorice, Catch Mini and Catch Dry Mini snus, respectively.

Conclusions:

The AUC of Catch Licorice and Catch Mini were very similar in spite of the twice as large dose of snus in Catch Licorice, 1 g versus 0.5g. The approximate relative bioavailable dose of each type of snus, based upon comparison to the AUC and bioavailable dose of Nicorette® 2 mg gum leads to an approximate bioavailability of 40% for General, Catch Mini and Catch Dry Mini, respectively, and approximately 60% for Catch Licorice portion snus.
The higher bioavailability found for Catch Licorice compared to Catch Mini may be due to more efficient absorption of the lower extracted dose from Catch Licorice, about 1.5 mg versus 2 mg of Catch Mini. It may be speculated that the reason for this is a saliva penetration factor, i.e. the doubled volume of snus in Catch Licorice compared to Catch Mini reduces the eluation of nicotine into the saliva.
Based on the results of the present study it may also be concluded that the risks of aggravation of heart failure and hypertension with respect to increased salt load from the use of snus are negligible.

1. ETHICS

1.1 Ethics review

The final study protocol, including the final version of the Subject Information and Consent Forms, was approved in writing by the Independent Ethics Committee at University Hospital, Lund, Sweden, before enrolment of any subject into the study. The principal investigator was responsible for informing the Ethics Committee of any amendment to the protocol as per local requirements. The investigator filed all correspondence with the EC. Copies of EC approvals were filed in the Master File that is forwarded to Swedish Match.

1.2 Ethical conduct of the study

The study was performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association Assembly, Helsinki, Finland, 1964 (Declaration of Helsinki) and later revisions. The trial was consistent with Good Clinical Practice (GCP) and applicable regulatory requirements.

1.3 Subject information and consent

The principal investigator ensured that the subject was given full and adequate oral and written information about the nature, purpose and possible risks of the study. The subjects were also notified that they were free to discontinue their participation in the study at any time. The subjects were given the opportunity to ask questions and time for consideration. The subject's signed informed consent was obtained before conducting any procedure specifically for the study. The original signed Consent Form was stored by the principal investigator.

2. INTRODUCTION

a. Background

The use of Swedish snuff (herafter snus) has increased steadily in Sweden while cigarette smoking has decreased, particularly among the men where the prevalence of smoking was 17% in year 2000. Only 10% of boys and 15% of girls 15 – 16 years old smoked (1). It is hard to estimate to what extent snus is responsible for the very low smoking prevalence in Swedish men. There are a number of factors that determine smoking prevalence, like attitudes to smoking, cigarette price, laws and regulations, awareness of the harmful effects, etc. It is hard to find any other factor that would have made Sweden so special, with 19% of the adult population smoking versus 31% in both of the neighbouring countries Norway and Denmark. This is by far the lowest prevalence of smoking in Europe. Among physicians the prevalence of smoking and snus use was 6 and 11 %, respectively, in 2001 (2).

Is snus an aid for stopping smoking? Fortyseven per cent of current snus users in 2001 were previous smokers according to a study commissioned by Swedish Match (3). Would they have managed to give up smoking if snus were not available? Most likely not. Among former smokers using snus there is a large fraction of highly nicotine dependent individuals. In a longitudinal study in southern Sweden it was also found that giving up smoking was associated with high snus use (4). Would part of the 53% that use snus that are never-smokers have smoked if snus had not been available? Maybe. If so, could there be a health benefit from the choice in favour of snus before cigarettes ? If snus were one tenth as harmful as smoking, it had to be used at least 10 times more to offset its benefit to public health (5).

In one study, commissioned by The Swedish Cancer Society and Pharmacia Corporation, where 1000 ex-smokers were asked about their quitting methods it was found that 50% had never used any help to stop, 33% had used snus and 17% nicotine replacement therapy (NRT). Twenty eight per cent of the men had used snus at the last quit attempt (6). It seems that highly dependent smokers, show the same probability of success as those with lower dependence when snus is used for quitting smoking (7). Usually the likelihood of cessation is directly related to the strength of the nicotine dependence (8). The explanation for the higher probability of success can be the relative similarity of the nicotine plasma concentrations obtained from snus and cigarette smoking (9).

The safety of snus. Every second life long smoker dies prematurely with a shortened life span by on average 6-8 years (10). The most common disease categories caused by tobacco smoking are cancer, respiratory and cardiovascular disorders. There are different types of smoke free tobacco (SFT), mainly wet (moist) snuff, e.g. snus, and dry snuff and chewing tobacco. Some SFT has been linked with some harm, e.g. oral cancer (11). Among SFT products reports of the risk of oral, pharyngeal and larynx cancer vary considerably. In a recent meta-analysis the highest risk was associated with use of dry snus and the lowest with moist snus that carried no increased risk (12). The Swedish snus is manufactured with close surveillance of possible carcinogenic substances, e.g. nitrosamines. Two Swedish case-control studies (13,14), showed no increased risk of oral cancer with snus use, while smoking and alcohol were associated with increased risk. The incidence of oral cancer in Sweden – the only country in Europe with considerable use of SFT – is among the lowest in Europe (15). Smoking but not snus use is shown to be associated with gastric cardia carcinoma and oesophageal carcinoma (16). Similarly, no increased risk for gastric cancer among snus users was found (17). In two other analyses of cancer at all sites no increased risk among snus users was found compared to non-tobacco users (18,19). A somewhat increased risk of myocardial infarction cannot be ruled out, however much smaller than the risk associated with smoking (20).

Except for Iceland, nicotine replacement products (NRT) have the highest penetration per capita in Sweden. In Sweden the switching from smoking to using snus has opened the eyes of the population to the fact that tobacco involves an element of nicotine seeking. This awareness may be one reason for the high use of nicotine replacement in Sweden. This high use of nicotine replacement may in turn partly explain the relatively low prevalence of smoking in females (21%), despite no substantial use of snus. This smoking prevalence compares very favourably with smoking in females in the neighbouring countries Norway (32%) and Denmark (29%).

b. Study rationale

The pharmacokinetic properties of American brands of oral moist snuff are studied (21). The pharmacokinetics of Swedish snus, however, is less studied. A documentation of the plasma nicotine levels and relative bioavailability following the use of snus therefore appears well motivated.

3. STUDY OBJECTIVES

The primary objective of the present study is to estimate nicotine plasma levels achieved following one day's regular use of four different types of snus compared with 2 mg Nicorette® chewing gum.

4. OVERALL STUDY DESIGN

The study had open label, randomized, four-way cross-over study. Due to low plasma levels of nicotine after single administration of the snus the study was performed during multiple dose conditions. Twelve male healthy regular snus users were given 12 hourly repeated doses of four different types of snus. The study also included the chewing of 2 mg polacrilex gum (Nicorette®) for reference. Thus the study comprised a total of five 12 hour sessions. A minimum period of 5 days was kept between the different sessions. Serial blood samples were drawn for determination of trough nicotine levels as well as nicotine levels during the last dosing interval. Descriptive statistics are used, thus no power calculation was included. Analysis of residual nicotine and residual sodium chloride in each dose of snus was performed. Calculations of extracted dose of nicotine and sodium chloride, respectively, from each type of snus were made. Serial venous blood samples were drawn for determination of plasma nicotine levels. Approximate relative bioavailable dose of each type of snus was calculated using the AUC of Nicorette® 2 mg gum as reference and the approximate 55% bioavailability found in the literature for most buccal nicotine preparations (22).

5. STUDY SITE AND TIMETABLE

The study was performed at the Clinical Trial Unit, Department of Clinical Pharmacology, University Hospital, Lund, Sweden, during October – December 2002. The analyses of plasma samples were performed at the ABS Bioanalytical Laboratories, London, England.

6. MATERIAL AND METHODS

6.1. Selection of study population

18-23 years old, male non-smoking healthy volunteers, regularly using 4 - 17 portions of snus daily since minimum 1 year were selected for participation in the study. They had no history of cardiac, kidney or hepatic disease, alcohol abuse or drug dependence. A physical examination including ECG and blood pressure showed no evidence of disease. No abnormalities were

found in a routine laboratory screening. As subjects were included they were given a number between 1 and 12. Each subject included in the study was uniquely identified by this number and the subject´s initials, which should appear on all study documents.

6.1.1. Screening phase/procedures

All subjects were after giving informed consent subjected to a health examination at Citykliniken, Lund, not more than three months prior to the start of the study. The following data were recorded at the pre-entry visit: date of birth, height and weight. A physical examination, including ECG, measurements of blood pressure (BP) and heart rate (HR) and a laboratory screen, was performed. The subject's medical and surgical history was also recorded. The subjects were tested for Hepatitis B and C and HIV before entering the study. The medical and drug histories included surgical or medical conditions which might interfere with absorption, distribution, metabolism or excretion of the drug. Since it is not possible to enumerate all of the conditions which might impair absorption, metabolism or excretion, the investigator should be guided by evidence such as:

- history of major gastrointestinal tract surgery such as gastrectomy or bowel resection.
- impaired liver function as indicated by abnormal liver function profile, e.g. elevated ASAT, ALAT, bilirubin.
- impaired renal function as indicated by abnormal creatinine values or abnormal urinary constituents such as albumin.

Blood chemistry, virology and hematology were performed at the University Hospital of Lund, Sweden. Urinalysis was performed by Citykliniken, Lund, Sweden.

6.1.2. Inclusion criteria

The following inclusion criteria needed to be fulfilled on study day 1:

1. Male non-smokers, 18 to 50 years of age.
2. Habitual use of ≥ 12 and ≤ 24 portions snus daily since minimum 1 year.
3. Healthy according to the health examination.
4. Written informed consent given.

6.1.3 Exclusion criteria

Subjects presenting any of the following criteria were to be excluded from the study at the pre-entry visit or at study day 1, as applicable:

1. Concurrent participation in another clinical trial.
2. History of allergy.
3. Donation of blood within 3 months prior to the start of the study.
4. Smoking and use of any other nicotine containing product during the last 12 hours preceding each study day.

6.1.4 Reasons for selecting the study population

This study was performed in healthy subjects in order to aid compliance with complex study procedures and to avoid interference with the study results from disease processes and other drugs. The inclusion and exclusion criteria were chosen in order to select subjects who were known to be free from any significant illness relevant to the proposed study. The study was restricted entirely to healthy male subjects. A subject was eligible for admission to study if inclusion criteria were fulfilled and if no exclusion criteria were present as verified by the investigator. The subjects were mainly students recruited from the University, Lund, Sweden.

6.1.5 Withdrawal of subjects from treatment or assessment

Subjects were free to discontinue their participation in the study at any time. If a subject decided to discontinue participation in the study, he was to be contacted in order to obtain information about the reason(s) for discontinuation and any adverse events. Whenever possible, the subject was to return for a clinical visit at the time of or soon after discontinuation. A subject could be withdrawn from the study at any time, at the discretion of the investigator.

6.1.6 Restrictions

The subjects were instructed to abstain from any form of nicotine for at least 12 hours prior to the drug administration, i.e. from 8 p.m. the evening before study days. Previous experience has shown that subjects that have abstained from smoking or use of snus 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. Water, coffee and other beverages were not allowed during the 30 minutes per hour when the subject kept snus in his mouth or chewed gum.

6.2. Study products

6.2.1 Study products

A. “General” 1 g portion snus containing 8.8$\pm$0.4 mg nicotine per portion.
B. “Catch” Licorice 1 g portion snus containing 7.0$\pm$0.1 mg nicotine per portion.
C. “Catch Mini” 0.5 g portion snus containing 4.5$\pm$0.3 mg nicotine per portion.
D. “Catch Dry Mini” 0.3 g portionssnus containing 4.8$\pm$0.6 mg nicotine per portion.
E. Nicotine gum (Nicorette®) containing 1.9$\pm$0.1 mg nicotine.

6.2.2 Randomization procedure

The investigational site was provided with a list with enrolment numbers. Before any assessments were performed for the purpose of the study, subjects were allocated enrolment numbers in consecutive order. The snus treatments were given according to a computer

generated randomization list in blocks of 4. The Nicotine gum (Nicorette®) was given on a separate day.

6.2.3 Packaging, labeling and storage

The various types of snus were delivered in their original packs as delivered from Swedish Match, Stockholm, Sweden. Individual packaging according to the computer generated randomization list was made at the Hospital Pharmacy, Helsingborg. Nicotine gum (Nicorette®) containing 2 mg nicotine was delivered in its original blister pack as available in the open market in Sweden from the pharmacy.
Labeling of each pack in Swedish was made by the Hospital Pharmacy. The bottles were labelled with the study code, study product, subject number, "For human pharmacological trial", dosage instructions, name of investigator, use before date, "Keep out of reach of children" and Croel, Lund.All study products were kept in a secure place under adequate storage conditions. The snus was stored in a refrigerator (+2 - +8 ºC). The chewing gum was stored at ambient room temperature.

6.2.4 Product accountability

The test articles were ordered by Croel HB, Helsingborg, Sweden. After packaging and labelling at the Hospital Pharmacy, Helsingborg, the investigational site was provided with the study products. The snus as well as the gum were delivered in their original containers. A "confirmation of receipt note" were completed. All unused test articles were returned at study termination to Swedish Match, Stockholm, Sweden, for analysis and destruction.

6.2.5 Selection of doses in the study

The aim of this study was to give doses that were not expected to cause any side effects and that did not, in any way, pose a hazard to the subjects' health. Furthermore, the doses were expected to give sufficiently high plasma concentrations to allow estimation of pharmacokinetic parameters with adequate accuracy.

6.3. Treatment regimens

The study products were given as multiple doses administered every hour from 8.00 a.m. to 7.00 p.m. (12 administrations). Only non-smoking personnel were allowed to perform practical functions in this study.

Snus was used under standardized conditions and executed as follows: One portion was placed and kept in the same place between the *upper* lip and the gum for 30 minutes.

Chewing of the gum was performed under standardized conditions and executed as follows: One piece of chewing gum was chewed every two seconds for 30 minutes.

6.4. Concomitant medication and treatment compliance

Medication, which was considered necessary for the subject's safety and well-being, could be given at the discretion of the investigator. The administration of all medication (including study drugs) had to be recorded in the appropriate sections of the Case Report Form (CRF).

There were no restrictions as to the use of OTC drugs, however the participants were requested to report such use, that was recorded on the CRF. No other drug under investigation was allowed concomitantly with the study drug. The subjects were not allowed to participate concurrently in any other study.

Treatment compliance: Each dose of the study products was administered at the investigational site under the supervision of the study nurse. The chewing of the nicotine gum was supervised by the study nurse. However, the chew intensity for each gum was difficult to supervise. The subjects were instructed to abstain from any nicotine containing products from 8 p.m. the night before each study day. However, violation of this rule could not be detected until post-study bioanalysis.

6.5. Blood sampling for determination of nicotine

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

before (0) and 1, 3, 5, 7, 9, 11, 11+10', 11+20', 11+30', 11+40', 11+50' and 12 hours after the first dose administration.

Only non-smoking personnel were allowed to perform practical functions in this study. The participating subjects were not allowed to assist in the blood sampling due to risk of contamination. The blood samples were left in ambient room temperature for not longer than 30 minutes and then centrifuged for 10 minutes at a relative centrifuged force (RCF) of 1000g at ambient room temperature. The plasma was transferred to plastic tubes, labelled with a unique sample number, and was stored frozen (-20°C) until analysis. The total amount of blood drawn from each subject (including samples for laboratory screening) during the whole study (about five weeks) did not exceed 325 ml. The plasma samples were shipped by courier door to door on dry ice to ABS Laboratories, London, England.

6.6. Bioanalysis of nicotine in plasma

The determination of nicotine was performed using capillary gas chromatography after a single liquid-liquid extraction of a basified plasma sample. A nitrogen selective detector provides high selectivity and sensitivity for the measurement of nicotine.

To quantify nicotine a multilevel calibration at seven concentrations was performed. The calibration line was fitted by means of a power curve fitting regression model using the equation $y=ax^b$. The samples were assayed once. If the sample showed concentrations considered by the Study Director to be outside those expected the sample was re-assayed. If

the repeat assay gave a result greater than ±10% of the first result a third analysis was performed, subject to the availability of sample. The limit of quantification was 0.5ng/ml. The analyses were performed at ABS Laboratories, London England.

6.7. Residual nicotine in used snus

Each used portion of the doses No 7-12 of snus of each preparation was placed in a sealed container, labelled with a unique number, frozen and stored at -20°C until analysed. Ten portions of unused snus of each preparation were also analysed. Mean nicotine content of these portions was used for the calculation of extracted dose of nicotine. Analysis of residual nicotine was performed at the Research Department, Swedish Match, Stockholm.

6.8. Residual sodium chloride in used snus

Each used portion of the doses No 1-6 of snus of each preparation was placed in a sealed container, and analysed for sodium chloride content. Ten portions of unused snus were also analysed. Mean sodium chloride content of these portions were used for the calculation of the extraction of sodium chloride. Analysis of residual sodium chloride was performed at the Research Department, Swedish Match, Stockholm.

6.9. Pharmacokinetic calculations

Medians of the multiple nicotine plasma analyses of each sample were used for all calculations. The pharmacokinetic calculations were carried out using the WinNonlin Standard® computer system for pharmacokinetic data analysis (Pharsight Corporation, Mountain View, CA 94040, USA). The maximum nicotine plasma concentration (C_{max}) and the time to peak plasma concentration (t_{max}) were determined from the observed plasma concentration-time curve after the last dose administration. The nicotine plasma concentrations were used for calculating the area under the plasma concentration-time curve of the last dosing interval (AUC_{11-12}) by the linear trapezoidal method.
Calculation of bioavailability: A calculation of the approximate bioavailability (F[%]) of nicotine from each type of snus was made by comparison of its AUC to the average AUC of Nicorette 2 mg gum and its extracted dose to the average extracted dose of Nicorette 2 mg gum, assuming the approximate 55% bioavailability found in the literature for most buccal nicotine preparations (22). The following formula was used:

$F = AUC_{snus}/\text{extracted dose} * 55/AUC_{gum} * \text{extracted gum dose}$

7 ASSESSMENT OF SAFETY

7.1 Description of Adverse Events

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. The outcome of the accident 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 health declaration) 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 is 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.

7.2 Adverse Event Reporting Period

The adverse event-reporting period for this trial begins upon receiving the first dose of investigational product and ends at the last administration of the reference.
All adverse events that occur in trial patients during the adverse event reporting period specified in the protocol must be reported to Swedish Match, 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 product should also be reported as an adverse event.

7.3 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.

7.4 Eliciting Adverse Event Information

The investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the trial subject. The question asked was “Have you noticed any symptoms since we asked last?”

7.5 Reporting

If a SERIOUS adverse event occurs, the Investigator is responsible for informing the Swedish Medical Products Agency (MPA). The CROEL monitor is to be notified by the investigator using the SERIOUS ADVERSE EVENT REPORT (SAER) form (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 MPA. 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.

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

REPORTING REQUIREMENTS FOR ADVERSE EVENTS

Gravity	Reporting Time	Type of Report
SERIOUS	Within 24 hours	Initial report on SAER
	Within 5 working days	Final report on SAER
NONSERIOUS	Per case report form submission procedure	Appropriate case report forms

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 first awareness of the adverse event.

7.6 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.

7.7 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 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 follow-up CRF.
Subjects who received at least one dose of the study drug should be included in all safety analyses.
Adverse events were classified according to the WHO Adverse Reaction Terminology (WHOART) and are reported in adverse event incidence tables. The incidence of adverse

events is summarized by (1) body system and preferred term; (2) dosing phase; (3) maximum severity; and (4) relation to study drug. Data from subjects with serious adverse events and from patients who discontinued due to adverse events are summarized and patient data listings are provided. Descriptive statistics are provided for adverse event results. Concomitant medications taken by patients are summarized by treatment period and drug class.

8 DATA MANAGEMENT AND STATISTICS

8.1 Randomisation

The four snus preparations were given according to a computer generated randomization list. The chewing gum was chewed on a separate occasion.

8.2 Data Management

Before data entry, the CRF's were checked for completeness and accuracy. Any missing data were completed, if possible. The bioanalytical data were compiled into a bioanalytical report by the responsible analyst. These data were used by the pharmacokineticist for the pharmacokinetic calculations (WinNonlin Standard® computer system©). The pharmacokinetic parameters were entered into a computer file by the pharmacokineticist. The responsible statistician transferred data from the computer files for a statistical analysis and compiled the result into a statistical report.

8.3 Statistical considerations

There was no formal statistical hypothesis testing performed. All analyses performed were exploratory and no conclusions from a statistical perspective were made. The analyses to be performed are descriptive, all variables which are continuous are presented using descriptive statistics such as (mean, std, median, min, max, etc.). Variables which are categorical are presented using frequency tables including number of observations and percent. The analyses are carried out both as a parallel group design and as differences within each patient.
The AUC and other variables measured over time are presented using graphs with the mean and standard error of mean plotted in the graphs.

All adverse events are presented by the different snus brands and if the amount of adverse events should be high, treatment emergent signs and symptom approach.

9 QUALITY CONTROL (QC) AND QUALITY ASSURANCE (QA)

Monitoring visits to the trial site were made periodically during the trial, to ensure that all aspects of the protocol are followed. The subject chart and other documents were reviewed for verification of agreement with data on Case Report Forms. The trial site was also

subjected to a quality assurance audit by the QA auditor. The investigator guaranteed access to CRFs, subject charts and all relevant documents by the monitor and the QA auditor.

The investigator and his personnel were available for questions at all monitoring visits and the QA audit. To enable inspections by regulatory authorities and/or independent audits, the investigator is keeping records of the identity of all subjects, sufficient to link e.g. CRFs and subject charts, original signed Informed Consent Forms, copies of CRFs and records of drug disposition. To comply with Swedish and international regulations, the investigator will retain the records until at least 15 years after study completion.

10 RESULTS

10.1 Demographics

Twelve male subjects, aged 18 to 23 years, using 4-17 portions of oral moist snuff per day were recruited. Ten subjects were ex-smokers, two subjects were never-smokers. One subject, No. 5, was excluded from statistical analysis due to baseline nicotine plasma concentrations exceeding 4 ng/ml. Data from his chewing gum session only were used. One subject's chewing gum session, No.12, was excluded from statistical analysis due to obvious non-compliance with the chewing instructions. Subject No.11 dropped out from the chewing gum session for personal reasons. Demographic data are shown in Table 1.

Table 1. Demographic data

Subject No.	Age	Height	Weight	Snus brand	No. Portions/day	Ex/Never smoker
1	20	184	71	General	10	Ex
2	20	174	85	General portion	17	Ex
3	19	183	75	Grov portion	13	Never
4	19	182	75	General portion	14	Ex
5	20	175	66	General	10	Ex
6	20	181	67	General portion	8	Ex
7	19	181	77	General portion	8	Ex
8	19	186	70	General	8	Ex
9	23	170	93	General portion	17	Ex
10	19	178	65	General	10	Ex
11	21	171	74	Göteborg Rapé	4	Never
12	18	184	70	General portion	17	Ex
Mean	19.8	179	74		11.3	
SD	1.3	5.3	8.1		4.2	

10.2 Nicotine extraction from snus and gum

The nicotine content (mean ± SD of 10 portions) of unused General snus was 8.84 ± 0.40 mg/ portion. The mean nicotine amount extracted, i.e. the difference between the amount of nicotine in unused and the residual amount in used General snus was 2.74±0.80 mg/portion. This corresponds to an average 31% of the dose. The inter-individual variation was

somewhat larger, SD=0.80 mg/portion, than the intra-individual variation, SD=0.57 mg/portion.

The nicotine content (mean ± SD of 10 portions) of unused Catch Licorice snus was 7.04 ± 0.12 mg/ portion. The mean nicotine extraction from Catch Licorice snus was 1.55±0.68 mg/portion. This corresponds to an average 22% of the dose. The inter-individual variation was about 50% larger, SD=0.68 mg/portion, than the intra-individual variation, SD=0.47 mg/ portion.

The nicotine content (mean ± SD of 10 portions) of unused Catch Mini snus was 4.53 ± 0.26 mg/ portion. The mean nicotine extraction from Catch Mini snus was 2.00±0.56 mg/portion. This corresponds to an average 44% of the dose. The inter-individual variation was about twice as large, SD=0.56 mg/ portion, as the intra-individual variation, SD=0.29 mg/ portion.

The nicotine content (mean ± SD of 10 portions) of unused Catch Dry Mini snus was 4.82 ± 0.58 mg/ portion. The mean nicotine extraction from Catch Dry Mini snus was 1.08±0.94 mg/portion. This corresponds to an average 22% of the dose. The inter-individual variation was about twice as large, SD=0.94 mg/ portion, as the intra-individual variation, SD=0.43 mg/ portion. Individual extraction is tabulated in Appendix 1.

The nicotine content (mean ± SD of 10 pieces) of unused Nicorette chewing gum was 1.91 ± 0.11 mg/ piece. The mean extracted amount, i.e. the difference between the amount of nicotine in unused and the residual amount in used Nicorette gum, ranged from 0.42 – 1.37 mg/piece (mean =0.84 mg/piece). This corresponds to an average 44% of the dose. The inter-individual variation was about three times larger, SD=0.34 mg/piece, than the intra-individual variation, SD=0.12 mg/piece. Individual extraction is tabulated in Appendix 1.

Table 2. Nicotine content in unused snus (Mean±SD of 10 portions)

Brand	Nicotine content (mg/portion)	
	Mean	SD
A GENERAL	8.84	0.40
B CATCH LICORICE	7.04	0.12
C CATCH MINI	4.53	0.26
D CATCH DRY MINI	4.82	0.58

Table 3. Nicotine extraction from snus (Mean±SD of subjects 1-12).

Brand	Extracted Nicotine (mg/portion)	
	Mean	SD
A GENERAL	2.74	0.80
B CATCH LICORICE	1.55	0.68
C CATCH MINI	2.00	0.56
D CATCH DRY MINI	1.08	0.94

10.3 Sodium chloride extraction from used snus

The mean sodium chloride amount extracted from General snus, i.e. the difference between the amount of sodium chloride in unused and the residual amount in used General snus was 8.13±7.33 mg/portion.
The mean sodium chloride extraction from Catch Licorice snus was 10.38±6.83 mg/portion. The mean sodium chloride extraction from Catch Mini snus was 5.58±4.49 mg/portion. The mean sodium chloride extraction from Catch Dry Mini snus was 4.73±6.61 mg/portion. Individual extraction is tabulated in Appendix 4.

10.4 Maximal nicotine plasma concentration

The mean C_{max} obtained in the last dosing interval after use of the General portion snus was 29.0 ± 8.5 ng/ml. The corresponding value after use of the Catch Licorice portion snus was 23.8 ± 8.6 ng/ml (Table 4).
The mean C_{max} obtained in the last dosing interval after use of the Catch Mini portion snus was 21.0 ± 6.9 ng/ml. The corresponding value after use of the Catch Dry Mini portion snus was 10.9 ± 5.7 ng/ml (Table 4).
The mean C_{max} obtained after chewing of the 2 mg nicotine polacrilex gum (Nicorette®) was 12.8 ± 4.7 ng/ml (Table 4).

10.5 Time to maximal plasma concentration

The median t_{max} in the last dosing interval after use of snuff was 30 minutes for all products. The median t_{max} after chewing of the 2 mg nicotine polacrilex gum (Nicorette®) was 30 minutes.

Mean plasma concentration-time curves are presented in Figures 1-4. Individual concentrations are tabulated in Appendix 2. "Spagetti plots" of individual plasma plasma concentration-time profiles are shown in Appendix 3. Deviations of actual from planned blood sampling time were negligible and are not reported.

10.6 Area under the plasma concentration-time curve

The mean AUC of the last dosing interval the last dosing interval after use of the General portion snus was 26.2 ± 3.4 h ∗ ng/mL. The corresponding value after use of the Catch Licorice portion snus was 21.6 ± 8.8 h ∗ ng/mL (Table 5).
The mean AUC of the last dosing interval after use of the Catch Mini portion snus was 19.0 ± 6.7 h ∗ ng/mL. The corresponding value after use of the Catch Dry Mini portion snus was 9.8 ± 5.1 h ∗ ng/mL (Table 5).
The mean AUC after chewing of the 2 mg nicotine polacrilex gum (Nicorette®) was 11.6 ± 4.5 h ∗ ng/mL (Table 5).

Figure 1.

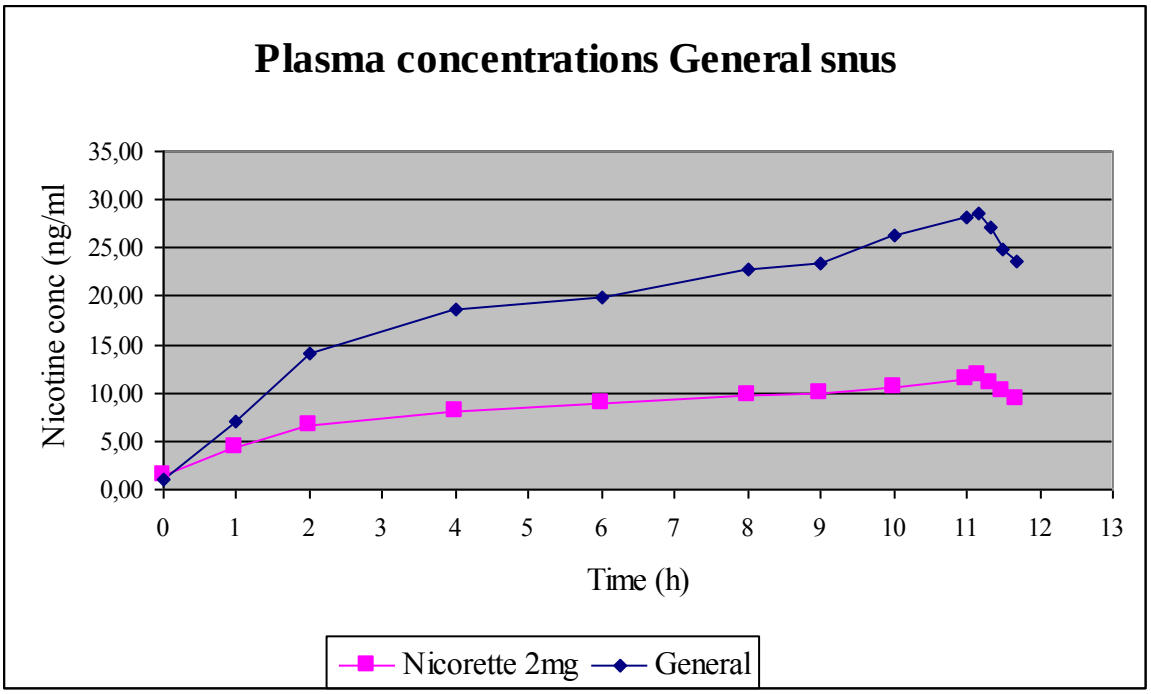


Figure 2.

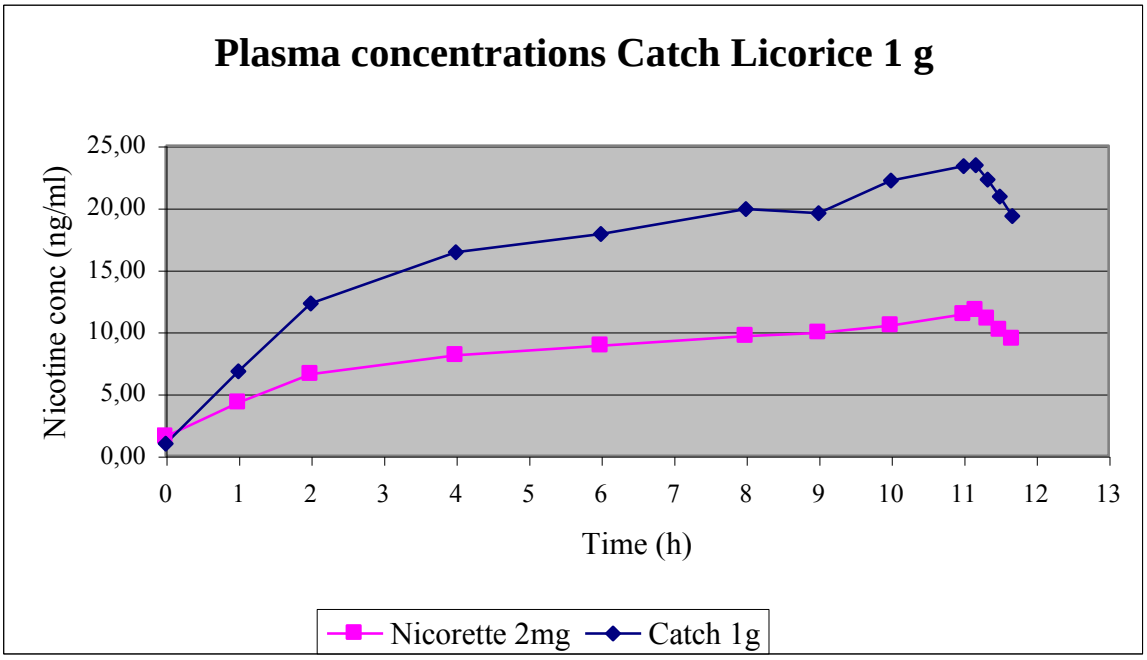


Table 4. Maximal nicotine plasma concentration

Subject No.	**Cmax** (ng/mL)				
	General	**Catch Lic 1g**	**Catch Mini**	**Dry Mini**	**Nicorette**
1	53.31	47.35	39.31	27.11	17.60
2	26.92	20.90	20.41	8.77	8.33
3	27.74	23.19	22.09	10.27	12.46
4	26.29	19.39	15.76	11.05	5.76
5*	-	-	-	-	-
6	23.09	15.46	15.64	8.75	7.18
7	28.76	24.63	15.54	7.25	18.23
8	31.52	23.40	21.96	8.67	11.63
9	23.39	15.51	15.48	7.74	12.10
10	22.60	16.95	20.21	6.79	15.89
11	25.89	24.52	25.14	10.80	18.40
12	29.46	30.41	18.88	12.20	2.26*
Mean	29.00	23.79	20.95	10.85	12.75
SD	8.53	8.60	6.90	5.65	4.67

*Excluded from analysis due to protocol violation.

Figure 3.

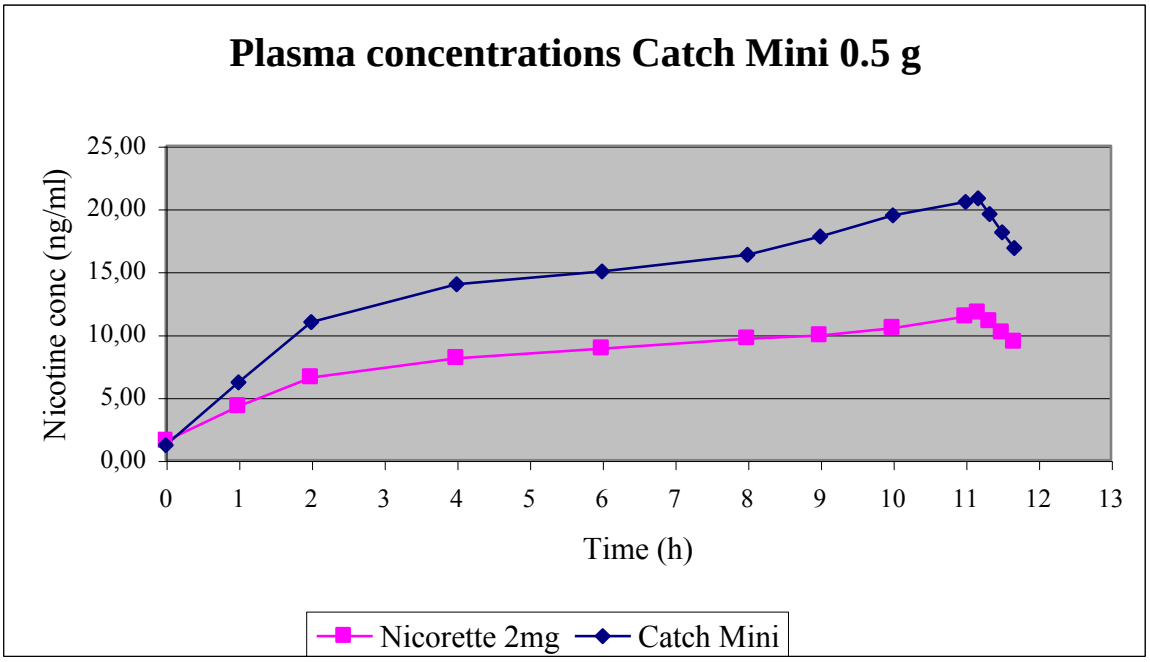


Figure 4.

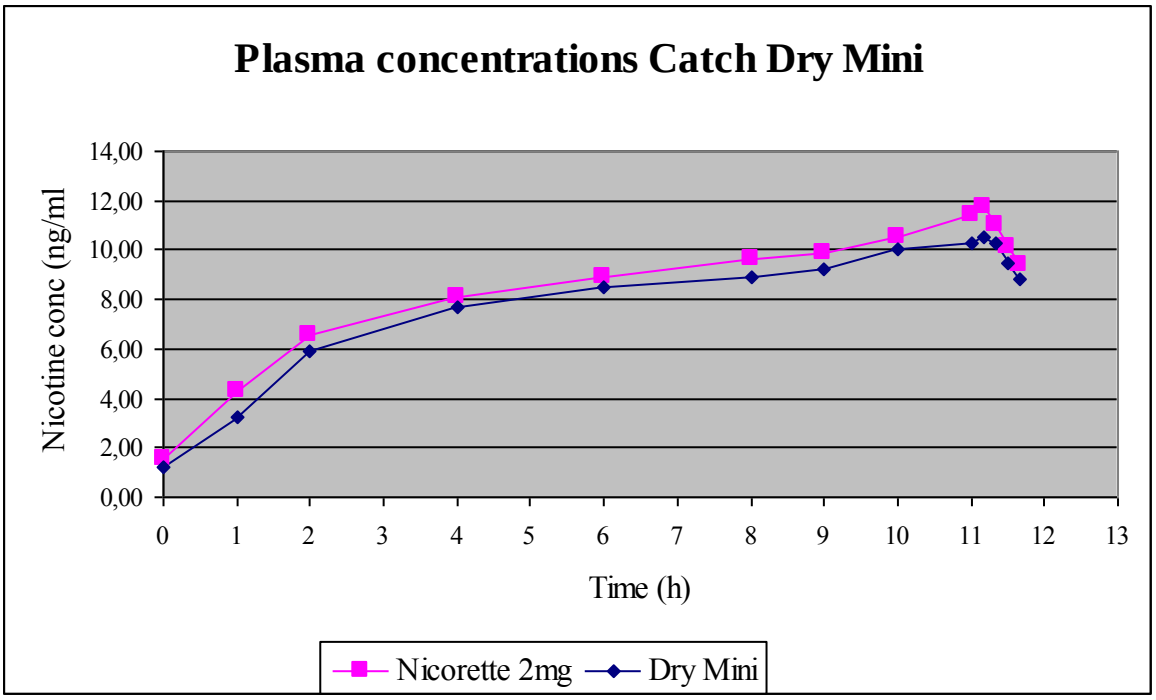


Table 5. Area under the plasma concentration-time curve (AUC).

Subject No.	**AUC** (h * ng/mL)				
	General	**Catch Lic 1g**	**Catch Mini**	**Dry Mini**	**Nicorette**
1	46.82	45.26	37.22	24.57	16.56
2	24.58	18.84	17.28	7.97	7.13
3	25.23	20.46	18.85	8.50	10.47
4	23.62	17.48	14.39	9.47	5.30
5*	-	-	-	-	-
6	21.50	14.34	15.11	7.82	6.40
7	24.20	20.94	14.70	6.73	16.68
8	29.09	20.63	20.29	8.15	10.24
9	20.65	13.76	13.78	7.00	10.59
10	20.89	15.37	19.34	6.40	15.00
11	23.40	22.54	22.92	9.74	17.14
12	27.79	27.67	15.34	11.51	2.15*
Mean	26.16	21.57	19.02	9.81	11.55
SD	3.36	8.82	6.69	5.12	4.52

*Excluded from analysis due to protocol violation.

10.7 C_{max} ratio of snus/ Nicorette® 2 mg gum

The mean±SD of the C_{max} ratios between the various snus preparations and the Nicorette® 2 mg chewing gum for the AUC are shown in Table 6. The mean C_{max} ratio versus Nicorette® 2 mg chewing gum for the General portion snus was 2.5 ± 1.0. The corresponding value for the Catch Licorice portion snus was 2.0 ± 0.7.

The mean C_{max} ratio versus Nicorette® 2 mg chewing gum for the Catch Mini portion snus was 1.8 ± 0.6. The corresponding value for the Catch Dry Mini portion snus was 0.9 ± 0.5.

Table 6. Ratio of C_{max} for the various snus preparations versus Nicorette® 2 mg chewing gum.

Subject No.	Cmax ratio			
	General	Catch Lic 1g	Catch Mini	Dry Mini
1	3.03	2.69	2.23	1.54
2	3.23	2.51	2.45	1.05
3	2.23	1.86	1.77	0.82
4	4.56	3.37	2.74	1.92
*5**	-	-	-	-
6	3.22	2.15	2.18	1.22
7	1.58	1.35	0.85	0.40
8	2.71	2.01	1.89	0.75
9	1.93	1.28	1.28	0.64
10	1.42	1.07	1.27	0.43
11	1.41	1.33	1.37	0.59
*12**	-	-	-	-
Mean	2.53	1.96	1.80	0.94
SD	1.01	0.74	0.60	0.50

Table 7. Ratio of AUC for the various snus preparations versus Nicorette® 2 mg chewing gum.

Subject No.	AUC ratio			
	General	Catch Lic 1g	Catch Mini	Dry Mini
1	2.83	2.73	2.25	1.48
2	3.45	2.64	2.42	1.12
3	2.41	1.95	1.80	0.81
4	4.46	3.30	2.72	1.79
*5**	-	-	-	-
6	3.36	2.24	2.36	1.22
7	1.45	1.26	0.88	0.40
8	2.84	2.01	1.98	0.80
9	1.95	1.30	1.30	0.66
10	1.39	1.02	1.29	0.43
11	1.37	1.32	1.34	0.57
*12**	-	-	-	-
Mean	2.55	1.98	1.83	0.93
SD	1.03	0.76	0.61	0.46

10.8 AUC ratio of snus/ Nicorette® gum

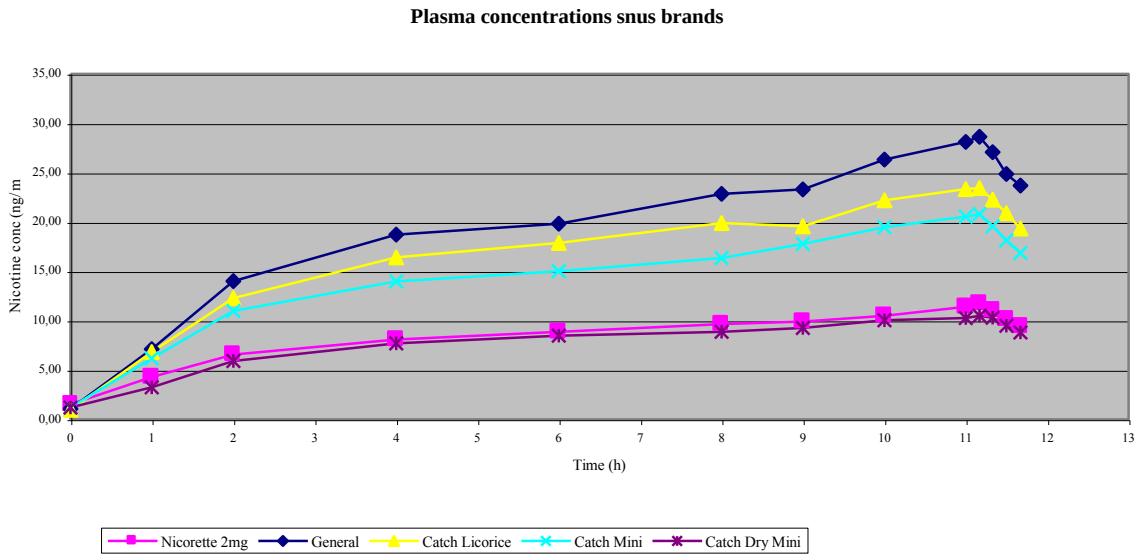
The mean±SD of the AUC ratios between the various snus preparations and the Nicorette® 2 mg chewing gum for the AUC are shown in Table 7. The mean AUC ratio versus Nicorette® 2 mg chewing gum for the General portion snus was 2.6 ± 1.0. The corresponding value for the Catch Licorice portion snus was 2.0 ± 0.8.

The mean AUC ratio versus Nicorette® 2 mg chewing gum for the Catch Mini portion snus was 1.8 ± 0.6. The corresponding value for the Catch Dry Mini portion snus was 0.9 ± 0.5.

11 Relative bioavailability

The mean plasma nicotine concentration-time curves for all brands of portion snus investigated show the relative size of the dose absorbed into the systemic blood circulation and may be compared with that of the Nicorette® chewing gum. See fig. 5. Approximate relative bioavailable dose of each type of snus was calculated using the AUC of Nicorette 2 mg gum as reference. An approximate 55% bioavailability is found in the literature for most buccal nicotine preparations. With the assumption of a 55% bioavailability of the Nicorette gum also in the present study, based upon the average extracted dose of 0.84 mg, the bioavailable dose of the gum is 0.46 mg. This figure used for the calculations should lead to an approximate bioavalability of 40% for General, Catch Mini and Catch Dry Mini portion snus, respectively, compared to 60% for Catch Licorice.

Figure 5.



12 Adverse events

All snus brands were very well tolerated and accepted. Adverse events were reported approximately 2-3 times per session for the Nicorette® chewing gum. Most frequently reported were hiccups, headache and irritated throat, with occasional reports of abdominal discomfort, cough and nausea. For the various brands of snus as well as for the Nicorette® chewing gum craving and withdrawal were reported in the morning hours of each session. With respect to craving and withdrawal General was most liked and Catch Dry Mini was least liked.

13 DISCUSSION AND CONCLUSION

The mean plasma nicotine concentration-time curve for the Catch Dry Mini portion snus showed great similarity to that of the Nicorette® chewing gum. See fig. 5. Catch Dry Mini was close to bioequivalent to the Nicorette 2 mg chewing gum. Catch Licorice 1g and Catch Mini 0.5 g were quite similar, with AUC and C_{max} twice those for Nicorette 2 mg gum. The AUC and C_{max} of General were 2 ½ times those for Nicorette 2 mg gum. Compared to smoking Catch Dry Mini once hourly produced blood levels similar to the lower end of cigarette smoking (7-10 cigarettes/day), while Catch Licorice and Catch Mini once hourly showed blood levels similar to moderate cigarette smoking (15-20 cigarettes/day). General once hourly produced blood levels similar to the upper end of cigarette smoking (25-40 cigarettes/day).

The AUC of Catch Licorice and Catch Mini were very similar in spite of the twice as large dose of snus in Catch Licorice, 1 g versus 0.5g in Catch Mini. The approximate bioavailable dose of each type of snus, based upon comparison to the AUC and bioavailable dose of Nicorette 2 mg gum leads to a 40% bioavailability for General, Catch Mini and Catch Dry Mini portion snus, respectively, compared to 60% for Catch Licorice. The higher bioavailability found for Catch Licorice compared to Catch Mini may be due to more efficient absorption of the lower extracted dose from Catch Licorice, about 1.5 mg (=22% of the dose) versus 2 mg (=44% of the dose) from Catch Mini. It may be speculated that the reason for this is a saliva penetration factor, i.e. the doubled volume of snus in Catch Licorice, compared to Catch Mini reduced the eluation of nicotine into the saliva.

The average sodium chloride amount extracted from each portion of the various brands of snus was approximately 7 mg, with General snus close to the average at about 8 mg and Catch Mini snus and Catch Dry Mini at about 5 mg. Catch Licorice showed the highest sodium chloride amount extracted, about 10 mg per portion. One tablespoon of salt corresponds to 6 g of sodium chloride, i.e. about 900 portions of snus. It may thus be concluded that the risks of aggravation of heart failure and hypertension with respect to increased salt load from the use of snus are negligible.

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