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**A study of the urinary cotinine in the employees
deriving from the exposure to nicotine at the
Nicorette patch and the Nicorette gum production
at Pfizer Health AB, Helsingborg**

Jarl P. Håkansson



Supervisor and tutor: Christian H. Lindh
Supervisor at Pfizer Health AB: Hans Thulin

Department of Occupational and Environmental Medicine
Institution for Laboratory Medicine
Lund University Hospital

Lund Institute of Technology
Lund University

Abstract

Air sampling and biological sampling have been performed to examine the exposure to nicotine in the Nicorette patch and Nicorette gum production at Pfizer Health AB in Helsingborg, Sweden. For this purpose stationary and personal sampling equipment have been used. At the Nicorette patch production, XAD-2 tubes were used to sample nicotine vapor and at the Nicorette gum production Teflon filters were used to sample the Nicotine Resin Complex 20% IRP (NRC) in the air. NRC is an ion exchange resin that carries the nicotine in the Nicorette gums. Specimens of urine from the presumably not exposed employees, from another source than at in the Nicorette production room, have been examined into the concentration of cotinine, a primary metabolite to nicotine. Cotinine is a reliable substance of measuring the exposure to nicotine.

For the nicotine vapor extraction, a slightly modified NIOSH's method NMAM-2544 has been used. The extraction of nicotine from the NRC and the Teflon filters was done with Pfizer's method NM-080-3 and an extra liquid-liquid nicotine extraction has been done as well. The same program, in a liquid chromatograph with tandem mass spectrometry (LC-MS/MS), was used for analyzing both the nicotine from the NRC and the nicotine vapor.

The specimens of urine were worked up and the urinary cotinine was isolated and transferred to an organic phase and analyzed in an LC-MS/MS.

The average concentration of nicotine in the ambient air in the Nicorette patch manufacturing room is low, $2 \mu\text{g}/\text{m}^3$. But at the reception position, where the employees collect the Nicorette patches, the concentration of nicotine is $30 \mu\text{g}/\text{m}^3$ on average. An average of the estimated nicotine exposure, based on the concentration of nicotine in the air samples for the employees, is $70 \mu\text{g}\cdot\text{h}/\text{m}^3$. It is also very obvious, from the urinary cotinine analysis, that there are persons who are exposed to more nicotine than from the nicotine vapor. This may be due to the fact that nicotine has penetrated through the skin when the employees have not used sufficient protection clothes.

In the Nicorette gum production room, the concentration of nicotine in the air for a whole working day is measured to an average value of $4 \mu\text{g}/\text{m}^3$, which is equal to $20 \mu\text{g}/\text{m}^3$ of NRC. The employees are exposed to the irritating NRC dust, which has on average a concentration of $35 \mu\text{g}/\text{m}^3$ of nicotine, which equals $175 \mu\text{g}/\text{m}^3$ of NRC. That gives an estimated average exposure of $90 \mu\text{g}\cdot\text{h}/\text{m}^3$ of nicotine, which equals $450 \mu\text{g}\cdot\text{h}/\text{m}^3$ of NRC, which comes from the nicotine measured in the air samples. The exposure is very well connected to the working procedures. In the Nicorette gum production site, the concentration of cotinine varied very much in relation to the amount of nicotine that the employees were exposed to from the air.

The NRC is very irritating and affects the upper respiratory region and causes coughs and sour throats. Irritation from the NRC is noticed in concentrations in the range from $13 \mu\text{g}/\text{m}^3$ up to $600 \mu\text{g}/\text{m}^3$. Measured in the concentration of nicotine, the range of the level, in which the irritation is noticed, corresponds to between 2.5 and $120 \mu\text{g}/\text{m}^3$. That is many times lower than the stated Threshold Limit Value (TLV) for nicotine, $500 \mu\text{g}/\text{m}^3$.

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A Introduction

A.1 Assignment

Pfizer Health AB, earlier Pharmacia and Upjohn has for the well-being and health of the employees and the environment tried to reduce sources of misconditions, pollutants and other factors that can cause any damage or injuries.

At the Nicorette patch and Nicorette gum production the employees are exposed to nicotine in the first phase of the manufacturing process. In the Nicorette patch production the exposure comes from nicotine vapor in the air and nicotine in the Nicorette patches, which are carried by hand. In the Nicorette gum production the exposure comes from the Nicotine Resin Complex 20% IRP (NRC). NRC is very dusty and irritates the employees when the NRC dust is inhaled in the production room. The NRC is used to carry unreacted nicotine and control the release of it when the Nicorette gums are chewed.

To examine the exposure of the employees, nicotine was sampled and measured in the air of the manufacturing rooms. The analysis of nicotine was done in a liquid chromatographic system with tandem mass spectrometric detection (LC-MS/MS). Cotinine was extracted from specimens of urine and analyzed in the LC-MS/MS, as well.

To be certain that the urinary cotinine comes from the exposure when manufacturing the Nicorette products, the employees had to be presumably not be exposed to nicotine during the study. A questionnaire was filled in every day during the participation in the study and the employees wrote down if they had been exposed to nicotine the days before and during their involvement in the research. In the questionnaire they also filled in their working tasks and the time used for the sampling of the nicotine.

Nine persons from the Nicorette patch production were participating in the study during three weeks. In the Nicorette gum production, seven persons participated for two weeks.

A.2 Aim

The aim of this diploma work is to study the exposure to nicotine for the employees in the production of Nicorette patch and Nicorette gum at Pfizer Health AB.

The study investigates the correlation between the nicotine content in the surrounding air and the urinary cotinine in both the Nicorette Patch and the Nicorette Gum production. The correlation may show if the exposure only comes from the air breathed, or if there is also the dermal route of exposure.

In the Nicorette Gum production, air samples of the NRC were investigated in order to determine the concentration that causes irritation in the respiratory region.

A.3 General information of nicotine

A.3.1 Exposure to nicotine

Nicotine has been used for a very long time as a drug and today tobacco is one of the most widely spread and used drugs in the world. Nicotine is an alkaloid found in many plants such as the potato, the tomato, the green pepper and of course the tobacco plants *Nicotiana tabacum* and *Nicotiana rustica*. The tobacco leaf usually contains from 1 to 3 % of nicotine. The biosynthesis takes place in the roots and nicotine is then accumulated in the leaves. [1, 2]

The most common ways of nicotine absorption are through the lungs, the skin and the oral cavity, but it can be absorbed, though very poorly, through the gastrointestinal tract and reabsorbed through the urinary bladder. Nicotine is dibasic and has two pKa of 3.04 and 7.84, at 25 degrees Celsius. The more basic a solution of nicotine, the more neutral the nicotine becomes and it can in a neutral state easily cross biological membranes. [2]

A.3.2 Metabolism of nicotine

Nicotine is metabolized in the body with phase I and phase II metabolisms. Phase I involves C-oxidation, N-oxidation and N-methylation. Phase II metabolism of nicotine involves O- and N-glucuronidation. Approximately 70-80% of the nicotine is phase I metabolized to cotinine in a C-oxidation, with the intermediary products 5'-hydroxynicotine and nicotine iminium ion. Nicotine metabolizes fast in the body, it has a half-life of 1-2 hours. Cotinine has a longer half-life than nicotine, 15-20 hours, and can therefore be used as a biomarker for nicotine exposure. The enzymes that are involved in the metabolism of nicotine to cotinine are the cytochrome P-450 (CyP) and aldehyde oxidase. Especially the enzyme CyP2A6 is involved in the metabolism of nicotine to cotinine. In humans there is a genetic polymorphism in the CyP2A6. The variations of the different CyP2A6 genes have shown different enzymatic activities. Cotinine and nicotine continue the formations of other substances, using the other phase I or phase II metabolisms. The different enzymatic activities affect the result of the total sum of the final molecules. For a human, the individual formation of cotinine varies and can differ even from the approximate formatted value. [2, 3, 4]

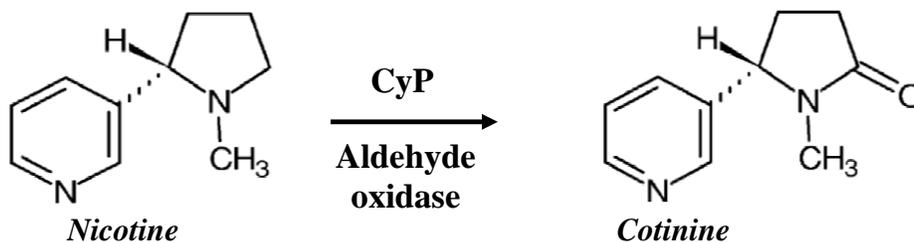


Figure A.1: Nicotine metabolizes by a phase I metabolism, C-oxidation, to cotinine, by the enzymes cytochrome P-450 and aldehyde oxidase in two steps.

A.3.3 Effects of nicotine

After nicotine has entered the body either through the lungs, the skin, the mouth or another way, it solves in the plasma and reaches the whole body quickly. Nicotine crosses the blood-brain barrier very easily, as well. Nicotine interacts with the nicotinic cholinergic receptors, which are found in the brain, the autonomic ganglia and in the neuromuscular junctions. In the brain there are different nicotinic receptors that vary in the agonist binding sites and the electrophysiological responses to stimulation. That can be one reason why the effects of nicotine vary. Prolonged exposure to nicotine gives more nicotinic receptors. The effect of an activation of the nicotinic receptor by nicotine is the release of neurotransmitters including acetylcholine, beta-endorphin, dopamine, glutamate, norepinephrine, serotonin and others. The addiction to nicotine that arises physiologically is strongly linked to the release of dopamine in the reward centers. [2, 3]

The effects of the nicotine vary depending on the dose of nicotine the exposure consists of. The effects are always very fast whether it is a low or a strong dose. A strong dose of exposure may cause a burning sensation of the upper respiratory tract, abdominal pain, nausea, vomiting and diarrhea. Other effects are headache, sweating, dizziness, hearing and sight disturbances, confusion, weakness and incoordination. The heart may beat irregularly or even stop. Trembling and convulsions, faintness, shortness of breath and collapse may occur, which can cause death from respiratory paralysis. [5] If a low dose of nicotine is used regularly (for example smoking) other effects will occur. Positive reinforcements like relaxation, improved concentration, improved cognitive functions and improved mood will appear. Side effects will drastically come as a result as the concentration of nicotine in the plasma lowers. The negative effects appear as symptoms such as nervousness, irritability, anxiety, impaired concentration and impaired cognitive functions. The nicotine increases the blood pressure and the heart rate too. [3]

After and during exposure to nicotine there is a development of tolerance. The dose to give the same effect from the nicotine must be stronger when the exposure is repeated. Tolerance develops as well as for other effects, like the cardiovascular and the toxic ones. The heart rate acceleration lowers when smoking regularly and the toxic effects (dizziness, headache and vomiting for example) disappear. [3]

Nicotine is very toxic in the cells. Studies have shown that nicotine in rats have resulted in less free radical scavenging enzymes, which are superoxide dismutase, catalase and glutathione reductase. A decrease in these enzymes results in increased generation of superoxide anion and hydrogen peroxide, which results in higher levels of hydroxyl free radicals. Other studies that investigated the influence nicotine on the apoptosis showed that overall the apoptosis was inhibited by nicotine. The effect from nicotine on the proliferation showed results that nicotine stimulates the proliferation. This potential together with the potential of the inhibition of apoptosis make the nicotine a candidate for being a carcinogen. In a couple of studies nicotine has even shown a genotoxic potential and a potential of affecting the gene expression. [2]

A.4 Analysis

Separations in a high performance liquid chromatograph (HPLC), like in other chromatographic separation equipments, use the equilibrium of the analyte between the mobile and the stationary phase. In the liquid chromatograph (LC), the mobile phase is a liquid. Different molecules and substances have different affinities to the mobile and to the stationary phases compared to the other substances. The more affinity the substance has for the stationary phase the longer time (retention time) it stays in the column. Because of the variations of time in the column, the different substances separate. The reversed phase LC has a water-based liquid and the stationary phase consists of hydrocarbon groups that are covalently bound to silica spheres. [6, 7]

The detection of the substances in a mass spectrometer (MS) is carried out first by the separation of a difference between the mass over charge (m/z) for the substances. From the column the mobile phase with analytes enters the MS. An ion source, electrospray ionization ionizes the analytes. They are ionized because of the high potential, several kilovolts, between the capillary needle at the end of the column and at the entrance of the MS. Small droplets are formed when the sample solution is pumped into the space (between the capillary needle and the MS) with atmospheric pressure where the electrical potential is. The mobile phase and the solvent are vaporized from the droplets, the more they vaporize, the higher the surface potential in the droplet gets. In the droplet the charge density increases due the decreasing size of the droplet, until the charge is too high for the surface tension to keep the droplet together. When the surface potential becomes too high the droplet explodes in a coulomb explosion¹. Smaller charged droplets are formed and this process continues until all of the mobile phase and solute have vaporized. [6, 7]

The analytes separate in the first quadrupole by the first m/z that is pre-adjusted. In the second quadrupole, nitrogen gas flows over the substances that have passed the first quadrupole. They collide with nitrogen gas and fragmentize. The third and last quadrupole has another m/z adjusted to separate one new fragment and only that fragment will pass through and reach the detecting instrument. The last fragmentation is characteristic for just that particular analyte, which is expected. [6, 7]

A.5 Measurement

To compare the concentration of nicotine from the air and the concentration of cotinine in the urine a few assumptions have to be made. First of all the individual amount of air inhaled differs a lot. A male person, when performing the work at the Nicorette patch and the Nicorette gum production inhales every hour between 0.5 m^3 and 1.5 m^3 of air and a woman at the same production site inhales between 0.4 m^3 and 1.3 m^3 of air per hour. The big differences are due to the individual body sizes, the varying ways of breathing, the amount of work performed that very hour or just specific work positions. [8]

¹ Coulomb explosion – a droplet's surface potential is higher than its surface tension, which makes the uniting forces of the droplet smaller than the forces repelling each other and thus the droplet bursts.

The other assumptions are that the metabolism of nicotine (chapter A.3.2) to the biomarker, cotinine, and the rate of the renal secretion of cotinine are the same for all the employees. Corrections to the concentration of cotinine can be done as well as to compensate for the dilution of the urine as for the rate of secretion.

Urine can be differently diluted. It varies because of varying water, food, coffee intake, exercise and many other factors. A correction to the density may therefore be necessary in order to measure and compare the unit of weight of the urinary substances instead of comparing their unit of volume.

Another correction for urine is the comparison to the concentration of urinary creatinine². Creatinine can be used as a comparable substance to other urinary substances. To use the urinary concentration of creatinine for an adjustment, the substance measured has to be secreted the same way as creatinine. For example food and exercise can effect the concentration of creatinine in the urine. Between the sexes there is always a difference, men secrete up to three times more creatinine than women. The urinary concentration of creatinine cannot be used without risks of errors and miscalculations of the results.

Studies of the half-life of cotinine in the body show that it is sometimes better to use the concentration of cotinine right as it is instead of correcting the concentration of creatinine or the urinary density [9].

A.6 Internal standard

The same amount of the specific internal standard (IS) is added to every unknown sample and every calibration standard sample of each of the three different analytical series³. For the analytical series of nicotine, tri-deuterium nicotine has been used as IS and for the analytical series of cotinine, the IS tri-deuterium cotinine has been used. The use of IS is done in order to compensate for the possible losses of material when the samples are prepared, as well as to make it possible to measure the right concentrations as the capability of the analytical apparatus is not good enough to give the exact reproduction results.

² Creatinine – creatine in muscles, for example, is degradable to its cyclic amide creatinine in the body at a constant rate. It is not reabsorbed in the tubuli of the kidneys and secreted by urine as urinary creatinine at a constant rate as well.

³ The analytical series are Nicotine vapour, Nicotine Resin Complex 20% IRP and Cotinine.

B Nicorette production

B.1 Nicorette patch

In the Nicorette patch production employees work in three shifts. The shifts are divided into five morning shifts, 6 a.m. to 2 p.m. from Monday to Friday. The working hours afternoon- and night shifts are from 2 p.m. to 10 p.m. or from 10 p.m. to 6 a.m. The afternoon- and night shifts extend from Monday afternoon or Monday evening to Thursday evening or Friday morning, four working days. When necessary for the production, weekends are also used to satisfy order intakes. The employees rotate their places in the shifts. Normally they work one week morning, one week afternoon and one week night, before they work the morning shift again. The rotation between positions during the week shift in the production of Nicorette patch is also done continuously, on a daily basis.

B.1.1 Exposure to the nicotine vapor

Exposure of the nicotine is only studied in the “coating room”⁴, because that is where the uncovered nicotine is handled. When the bottle of liquid nicotine is handled a mask with airflow over the face, is worn. An apron and extra thick protection gloves, of plastic rubber are also worn during this moment. The 1-liter-bottle with nicotine is put into a box with a constant nitrogen gas flow that keeps the nicotine vapor from entering the production room. The presence of the nitrogen gas also makes sure that the oxygen contents inside the box remain low.

Nitrogen overpressure is present in other parts of the manufacturing process to keep out oxygen. The free nicotine easily oxidizes in air. Other parts in the production are not fully covered from the surrounding room. Consequently nicotine vapor can easily enter the coating room.

The employees are exposed to nicotine when they perform other tasks as well; most of the exposure comes from when they receive the Nicorette patches, at the reception position (picture B.1). At the reception an employee collects the Nicorette patches and stacks them on a



Picture B.1: The Mark Andy in the coating room, at the Nicorette patch production, with the view from the reception position. Stationary sampling was done right over this position in the breathing zone.

⁴ The coating room – the room in the Nicorette patch production where Nicorette patch is manufactured in the Mark Andy machinery.

perforated bench, with air extraction. Another employee carries the stacks of Nicorette patches by hand from the reception at the Mark Andy⁵ (picture B.1) machinery to another air extracted, perforated bench. On that bench the Nicorette patches are marked and wrapped in aluminum foil.

Overall ventilation is run all the time, three large overall air extraction regulators are situated in the ceiling just above the Mark Andy. If the ventilation is interrupted or drastically lowers warning indicators give notice.

During these moments in the production of Nicorette patch, the LIF-clothes⁶ and double layers of protection gloves are worn. [10]

B.2 Nicorette gum

The production in the Nicorette gum manufacturing as well as in the Nicorette patch production is divided into shift work. The shifts are divided into three, two shifts daytime and a constant night shift. The morning shift extends from 6 a.m. until 2 p.m., Monday to Friday, the afternoon shift, Monday to Thursday 2 p.m. until 10 p.m. and the night shift starts after the afternoon shift Monday night for eight hours and finishes Friday morning 6 a.m. Production does often take place during weekends too, if order intakes make it necessary. The employees have one morning shift and one afternoon shift every second week. The employees that work night shift always work night. Like in the Nicorette patch production, the changing of working positions is also done continuously, from day to day, to vary the workload during the week shift.

The mixing of NRC is done in a cupboard behind a transparent plastic door and with the use of a pair of thick plastic protection gloves, a glove box, see picture B.2. The design of the glove box makes it possible to handle the open bag containing the NRC safely. Inside the glove box there is a perforated steel bench



Figure B.2: The glove box where the NRC is filled into the container below. The glove box has a perforated bench with air extraction.

⁵ Mark Andy – the brand of the machine that processes the manufacturing of the Nicorette patch.

⁶ LIF-clothes are protection clothes that are used in the clean production area. The LIF-clothes consist of a cap (single use) which covers hair and ear, a vest, trousers and socks that are used up to one day, depending of the contamination, and slippers.

with air extraction. There is a big hole in the bench, which leads down into the container for mixing the NRC. Between the container and the bench there is a sieve, which prevents the powder from clustering.

The room where the premix, the mixing room⁷, is manufactured is ventilated with a few point extractions in the walls and four large overall air extractions in the ceiling. There are two central vacuum cleaners in the room for cleaning.

B.2.1 Exposure to Nicotine Resin Complex 20% IRP

Exposure to the NRC occurs during the preparing of the premix batch⁸ of Nicorette gum. In this mixing activity the components, which are also in powder condition, fill the room, which becomes more or less dust-laden.

When the container below the sieve is filled with the NRC, it is made to only contain the NRC, it dusts and the exposure to the NRC occurs. Other exposure occurs when the NRC

is emptied into the premix container (picture B.3). Because of the NRC's tendency to dust when emptied into the premix-container, consequently the less content in the container the less dust. Although most of the employees use the container for filling it with the other Nicorette gum ingredients, sodium carbonate, sodium bicarbonate, acesulfame K and magnesium oxide, with the NRC. Then the room quickly becomes very dust-laden and severe irritation occurs.



Figure B.3: The premix container where the Nicorette gum ingredients, in powder condition, are mixed.

Very rarely exposure of the NRC occurs when the premix is emptied into the warm chamber, where the Nicorette gum components, including the liquid menthol are mixed together. All of the gum components above mentioned and xylitol, may dust through the not totally sealed joint, between the

⁷ The mixing room – the room where the components of the Nicorette gums are mixed together to the premix batch.

⁸ Premix batch – the result from the first mixing of the Nicorette gum powder ingredients; acesulfame K, magnesium oxide, Nicotine Resin Complex 20% IRP, sodium bicarbonate and sodium carbonate.

premix container and the tube to the chamber below. The following mixing process takes place at the floor below the mixing room, where no exposure to nicotine occurs.

The employees wear the LIF-clothes and as usual when handling the NRC, a mask⁹ as well.

C Methods

C.1 Nicotine

C.1.1 Nicotine vapor

C.1.1.1 Nicotine vapor sampling procedure

The air sampling was done with nicotine absorbent, XAD-2 sorbent sample cartridges (SKC Tube XAD-2), containing an Amberlite resin, which easily absorbs organic compounds. The XAD-2 cartridge was attached to an air-blowing pump (MSA, Escort ELF Pump, Aver or SKC, Aircheck sampler model 224-52) with a rubber hose (Latex tubing, Amber, VWR Scientific).

The XAD-2 cartridges were attached at the level of breathing, the breathing zone¹⁰, on the shoulder of the employees. The pumps, which suck around one liter of air per minute through the XAD-2 tube, were running while the employees worked inside the coating room.

Other pumps were installed in the room at different places to measure the amount of nicotine content in the ambient air in the coating room. One pump was placed right over the bench where the Nicorette patches were received and collected. Another pump was placed either by a wall a couple of meters away from the Mark Andy, or it was placed by the doors of the room, as well as a couple of meters from the Mark Andy. One measurement was done in direct contact with the Mark Andy, by the waste roll. All the XAD-2 cartridges attached to these pumps were placed in the breathing zone. All sampling was done with an air-blow of around one liter per minute. (*Figure A.5.1 – Map of the coating room, Appendix 5*)

The air samples that were collected in the coating room for Nicorette patch, were marked and stored for a maximum of 4 weeks at -20 degrees Celsius, depending on when the samplings were done.

C.1.1.2 Extraction of nicotine from the XAD-2 and sample preparation

First the cartridges were sawed and cracked to collect the glass wool and the Amberlite resin. Nicotine was extracted from the Amberlite resin with ethyl acetate (Lab-Scan Analytical Sciences) [11]. The glass wool and the resin were put into 5 ml glass test

⁹ Mask – the mask is a mask covering the mouth and nose made to protect the respiratory region from dust.

¹⁰ Breathing zone – the area around the head of a person standing in an upright position.

tubes. 1.9 ml of ethyl acetate and 0.1 ml of internal standard (IS) tri-deuterium nicotine (Lab-Scan Analytical Sciences) in ethyl acetate with the concentration of 10 µg/ml, were added. The test tubes were shaken vigorously for 30 minutes and afterwards the solute was collected with a glass pipette and put into vials for the liquid chromatograph tandem mass spectrometry (LC-MS/MS) (Perkin-Elmer Biosystems, PE SCIEX API 3000, LC/MS/MS System, Triple quadrupole LC-MS/MS mass spectrometer). The vials were kept in a freezer at -20 degrees Celsius until analysis. Prior to the analysis all vials were centrifuged to make sure that there were no rests of glass wool in the analyte solute.

C.1.1.3 Preparation of calibration standard series for the vapor samples

Nicotine (Janssen Chimica) was weighed two times to make two separate calibration standard series. They were diluted in ethyl acetate, into concentrations from 12 ng/ml to 13 µg/ml, to make two calibration standard series, each with seven points, from 0 ng/ml to 13 µg/ml. Then 1.9 ml of the different solutes with nicotine was put in a 5 ml glass test tube together with 0.1 ml of the IS described in E.1.1.2. The test tubes were shaken for 30 minutes and the solute was transferred to a vial suited for the LC-MS/MS. The vials were stored until analyze in the freezer at -20 degrees Celsius.

C.1.2 Nicotine Resin Complex 20% IRP

C.1.2.1 Nicotine Resin Complex 20% IRP sampling procedure

Sampling the NRC from the air in the Nicorette gum production was done with a Teflon filter (Gelman Sciences, Teflon TF 1000, diameter 37 mm, pore size 1 µm), placed in a filter cassette (Omega Specialty Instruments Co., filter cassettes 37 mm). The cassette was then attached to a rubber hose, which was attached to the pump.

Samplings were done in different manners. One was personal air sampling, another was stationary area air sampling during a whole working day and the third was to measure the level of NRC when irritation¹¹ is noticed. The third mode of sampling was divided into three parts; “before – *no*”, “before and after – *no and after*” and “after – *after*” notice of irritation of the NRC, which was present in the ambient air. In the first two mentioned sampling modes around one liter of air was blown through the filter. In the latter three sampling modes, two liters of air were sucked through the filter. All sample equipment collected air in the breathing zone. (*Figure A.5.2 – Map of the mixing room, Appendix 5*)

After sampling, the Teflon filters were collected from the filter cassettes and placed in a 5 ml test tube; the tubes were marked and kept at -20 degrees Celsius until preparation.

C.1.2.2 Extraction of nicotine from the Teflon filters and sample preparation

Nicotine was extracted from the Teflon filter with ammonium hydroxide. The cat ion of nicotine in the resin was neutralized with 1 M ammonium hydroxide. The 1 M

¹¹ Irritation – the irritation from the Nicotine Resin Complex 20% IRP gives cough, sour and irritate throat.

ammonium hydroxide was prepared from 25% ammonia solution (Merck Eurolab AB) and milli-Q water (at 18.0 MΩ) in proportions 7.5 to 100 [12]. Then 3 ml of 1 M ammonium hydroxide was added to the 5 ml test tube containing the sample Teflon filter. The test tubes were shaken vigorously for 20 minutes. Afterwards centrifugation was done for 12 minutes at 2500 rpm, and 2 ml of the clear solution were transferred to a 13 ml test tube.

The solved nicotine in the ammonium hydroxide was extracted liquid to liquid, to the ethyl acetate. After this 1.9 ml of ethyl acetate and 0.1 ml of the IS (10 µg/ml tri-deuterium nicotine in ethyl acetate) was added to the sample solution of ammonia hydroxide. The test tubes with the mixture were shaken vigorously for 25 minutes. To make the two phases separate completely, the test tubes were centrifuged at 2000 rpm for 10 minutes. The supernatant was collected and put into a vial for analysis. Prior to the run in the LC-MS/MS apparatus, the vials were centrifuged. Until analyze, the samples were kept in the freezer at -20 degrees Celsius.

C.1.2.3 Preparation of calibration standard series for the NRC samples

For the calibration standard curves nicotine was weighed two times for two standard curves. The nicotine was diluted with 1 M ammonia hydroxide into concentrations of 0.3 ng/ml to 20 µg/ml. The solutes of nicotine in ammonia hydroxide in each calibration standard series had 8 points. 2 ml of the calibration standard solute was put into a 13 ml test tube with 1.9 ml ethyl acetate and 0.1 ml IS tri-deuterium nicotine (the same IS used in the preparation of the samples in C.1.2.2). The test tubes were shaken vigorously for 25 minutes. After that the test tubes were centrifuged at 2000 rpm for 10 minutes. The supernatant was transferred to a vial for the analysis in the LC-MS/MS. The calibration standard series were kept together with the unknown samples in the freezer, at -20 degrees Celsius, until time for analyze.

C.1.3 Analysis of nicotine

The separation procedure in the nicotine analysis is a short gradient separation (*see step table A.4.1, Appendix 4*). The mobile phase consists of a water based phase (10 mM ammonium acetate (Merck Eurolab AB), pH 9.5) – phase A, and an organic phase (methanol (Lab-Scan Analytical Sciences)) – phase B. First the nicotine is transported into the column (Agilent Technologies, Zorbax Extend-C₁₈, 2.1 * 50 mm, 3.5 µm) with the mobile phase in the volume-to-volume ratio 99% of A to 1% of B. Because of the high concentration of the water phase in the mobile phase, the nicotine will have a strong affinity to the stationary phase. When the methanol enters the column in a higher concentration, 95% of B to 5% of A, the nicotine will disperse to the mobile phase much better. The nicotine will then elute in a high concentration. The ammonium acetate must be present in the mobile phase when nicotine is eluted. It is difficult to run nicotine in a chromatographic system, because of its tendency to interact with the stationary phase. The pH in the water phase is very important for the concentration of nicotine during the elution. Nicotine has a very strong power of sticking to the stationary phase. As an effect

of this strong reactivity the following negative results can be tailing tops¹² or carry-over effects¹³.

The analytes are ionized with electrospray ionization when entering the MS. The MS was adjusted to select the nicotine with the m/z 162.9 atomic mass units (amu), in the first quadrupole. The third quadrupole had the adjustments for the fragments of the m/z 130.1 amu and also the m/z 84.3 amu. For the calculation of the concentrations of nicotine the m/z 162.9 and 130.1 were used. For the IS of tri-deuterium nicotine, the first quadrupole was adjusted to the m/z 166.0 amu and after the fragmentation the third quadrupole was adjusted to the m/z 87.3 amu.

C.2 Cotinine

C.2.1 Specimens of urine

C.2.1.1 Urine sampling procedure

To analyze the exposure to nicotine, urinary cotinine was used as its biomarker. The test persons gave a zero-sample, a specimen of urine, the morning of the same day the exposure to nicotine was expected. Then they gave a specimen of urine the morning after participating in the sampling of nicotine in the air inside the production rooms.

The specimens of urine were collected on the same day as they were given and kept frozen at -20 degrees Celsius until preparation. They thawed in 4 degrees Celsius over night before preparation.

C.2.1.2 Isolation of cotinine from the specimen of urine and preparation of calibration standard series

A specimen of urine was collected from a person who had no known exposure to nicotine for at least one and a half weeks. This sample was used for the calibration standard preparation. The importance of the non-exposed person is that the sample does not contain any cotinine.

Cotinine (Sigma Chemical Co.) was weighed two times to make two calibration standard series. Then the two sample series were diluted with methanol into concentrations from 0.3 ng/ml to 4 µg/ml.

50 µl and 100 µl of the calibration standards were applied together with 100 µl of the internal standard tri-deuterium cotinine (Sigma Chemical Co.) of 200 ng/ml to 1 ml of the urine in a 13 ml glass test tube. The concentration of cotinine in the solutions provide two

¹² Tailing top – the highest concentration of the analyte comes to the detector first. Because of the analyte's high affinity to the column's stationary phase some of the analyte elutes with longer retention time.

¹³ Carry-over effect – some of the analyte can stick to the injection needle, column or other parts of the analytical instrument, that may result in the wrong analyze-result in the following analyze run. The result in the former run may be too small and the result of the latter run may be too big.

standard stocks, each with nine points, from 0 (blank¹⁴) to just above 200 ng per ml of urine, every calibration standard has 20 ng of the IS tri-deuterium cotinine per ml urine as well. The method used to isolate the cotinine from the specimen of urine has the same procedure as the method for the preparation of the calibration standard. 1 ml of the urine from the specimens of urine was mixed together with 100 µl of the IS tri-deuterium cotinine in a 13 ml glass test tube.

To each unknown sample of urine and each stock of calibration standard, 7 ml of ethyl acetate and 1 ml of 5 M potassium hydroxide (Merck Eurolab AB) were added. The samples and the calibration standard stock solutions were then shaken vigorously for 15 minutes and the test tubes were centrifuged at 2000 rpm for another 15 minutes. The organic supernatant was collected and transferred to another 13 ml test tube of glass. The test tubes were then placed in the evaporator for around 45 minutes at 45°C. After evaporation, 100 µl of methanol was used to resolve the cotinine and the internal standard in the test tubes. The solute was transferred into a micro-vial, which was placed inside a vial, positioned in the liquid-chromatographic instrument for analysis. [13]

Because of the large amount of samples, they were prepared on different occasions, for example when a new set of samples was prepared and when fresh calibration standards were prepared.

C.2.2 Analysis of cotinine

The analyzing process of cotinine in the LC-MS/MS is done with a gradient (*see step table A.4.3, Appendix 4*). First the mobile phases, 95% of water based phase (10 mM ammonium acetate, pH not adjusted – phase A) and 5% of organic phase (methanol – phase B) transport the cotinine and other substances to the column (Agilent Technologies, Zorbax C18, 4.5 * 50 mm, 5 µm). The gradient changes the concentrations to 5% of the water phase and 95% of the organic phase for four minutes. The cotinine will then elute sharply, in a very high concentration. After four minutes the concentrations return to their initial values, for six seconds, and the initial concentrations last for the rest of the analytical program. [13]

In the entrance of the MS the analytes are ionized with electrospray ionization. In the analysis of cotinine, the first quadrupole of the MS was adjusted to the m/z 177.0 amu for cotinine and m/z 179.0 amu for the IS of tri-deuterium cotinine. The third quadrupole was adjusted to m/z 80.1 amu and m/z 98.2 amu to select the fragments from cotinine and the m/z 80.3 amu for the IS.

C.2.3 Density of urine and urinary concentration of creatinine

The density of urine is measured with a refractometer¹⁵ (Hand Refractometer, Ladassco ATAGO). The measurement of the concentration of creatinine has been performed by the Laboratory of Clinical Chemistry and Pharmacology at the University Hospital in Lund.

¹⁴ Blank – a blank sample does not contain any of the substances analysed, but the internal standard is usually present.

¹⁵ Refractometer – an instrument that measure the angle of refraction of a liquid.

C.3 Exposure calculation

The concentration of nicotine has been calculated using Calculation 1 or 2 (*Appendix 6*), depending on, in which production room the measurements have been done. The individual exposure has been estimated as the concentration of nicotine in the air for the employee multiplied by the rate of breathing, multiplied by the time working in the production room (*Calculation 3, Appendix 6*).

C.4 Quantification

The quantifications of the different analytes were done by using standard calibration curves from the standard series of analytes prepared from nicotine and cotinine. In every standard series sample a specific and identical amount of the internal standard, either tri-deuterium nicotine or tri-deuterium cotinine, was added. The points of the standard series samples weighed concentrations were plotted to the results from the LC-MS/MS. The results from the LC-MS/MS were the areas from the analyte (nicotine or cotinine) divided to the area of the internal standard. A standard calibration curve was fitted to the points.

To receive the concentration of the unknown samples, a calculation based on the function of the standard calibration curves was used. The analyte's area results divided by the internal standard results from the unknown samples were fitted into the function and the concentrations were calculated.

The limit of detection was estimated according to the concentrations of the calibration standard series for the different analytical series.

The analytical series of nicotine were prepared differently and consequently the concentration of tri-deuterium nicotine varied in the different analytical runs and the calibration curves got different gradients.

D Results

D.1 Nicotine

D.1.1 XAD-2 tubes

The limit of detection, in the LC-MS/MS system, for the concentration of nicotine vapor, from the extraction of the XAD-2 tubes, is estimated to 10 ng/ml.

The peak in figure D.1 shows the chromatogram of nicotine absorbed in the first layer of sorbent – A – in one of the XAD-2 tubes. Figure D.2 shows the chromatogram of the internal standard tri-deuterium nicotine, applied to the sample shown in figure D.1.

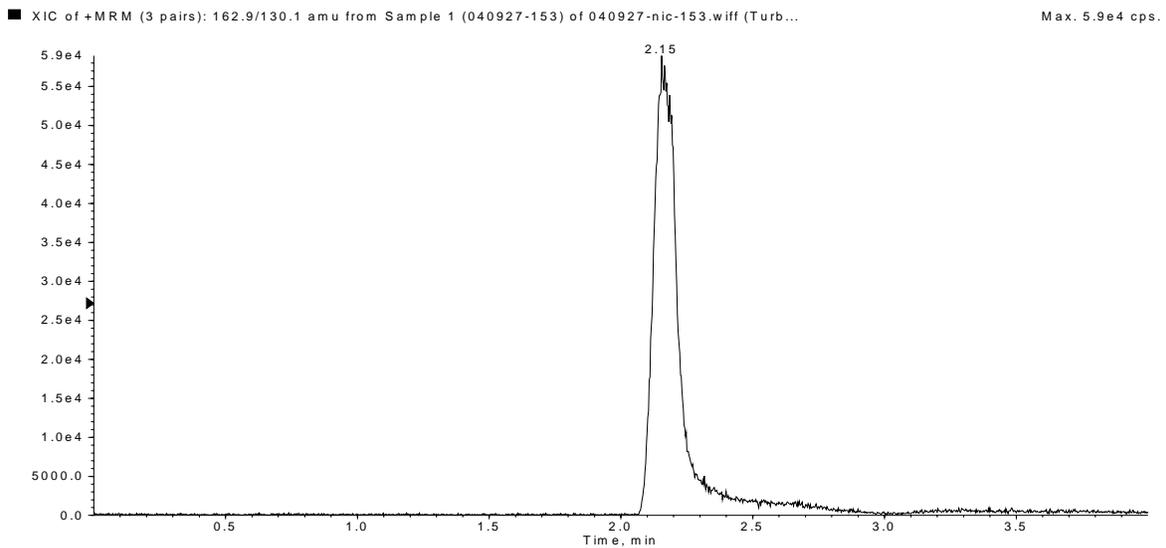


Figure D.1: Nicotine in the air in the Nicorette patch production, XAD-2 layer A.

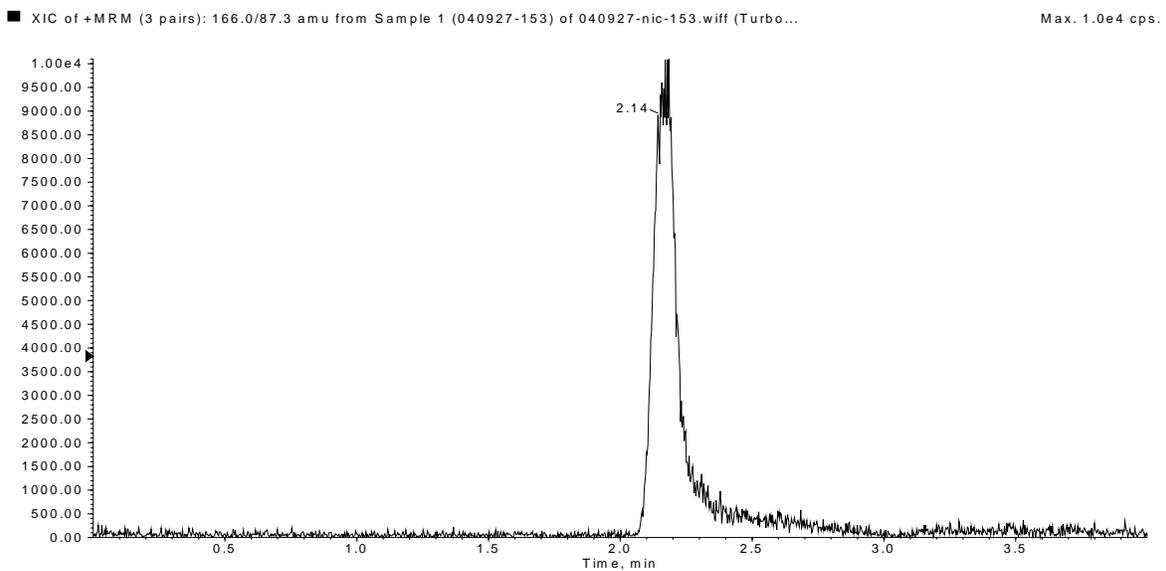


Figure D.2: Internal standard tri-deuterium nicotine in the sample of figure D.1.

The second sorbent layers – B – were run separately from the first layers – A. Figure D.3 shows the chromatogram of nicotine in the layer B and figure D.4, the chromatogram of the internal standard tri-deuterium nicotine.

■ XIC of +MRM (3 pairs): 162.9/130.1 amu from Sample 1 (040927-154) of 040927-nic-154.wiff (Tur... Max. 1760.0 cps.

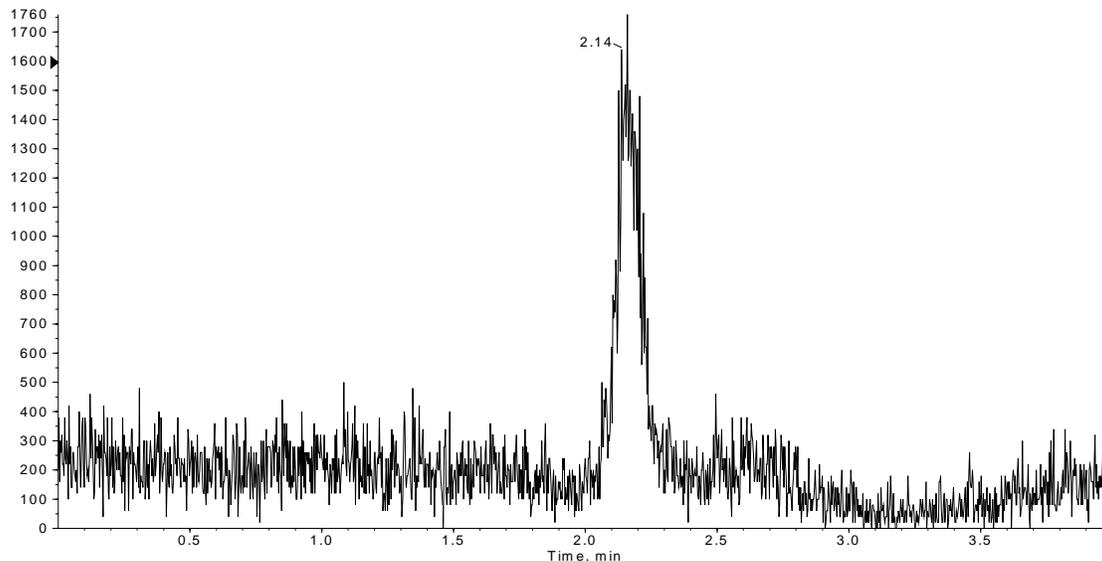


Figure D.3: Nicotine in the air in the Nicorette patch production, XAD-2 layer B.

■ XIC of +MRM (3 pairs): 166.0/87.3 amu from Sample 1 (040927-154) of 040927-nic-154.wiff (Turb... Max. 5100.0 cps.

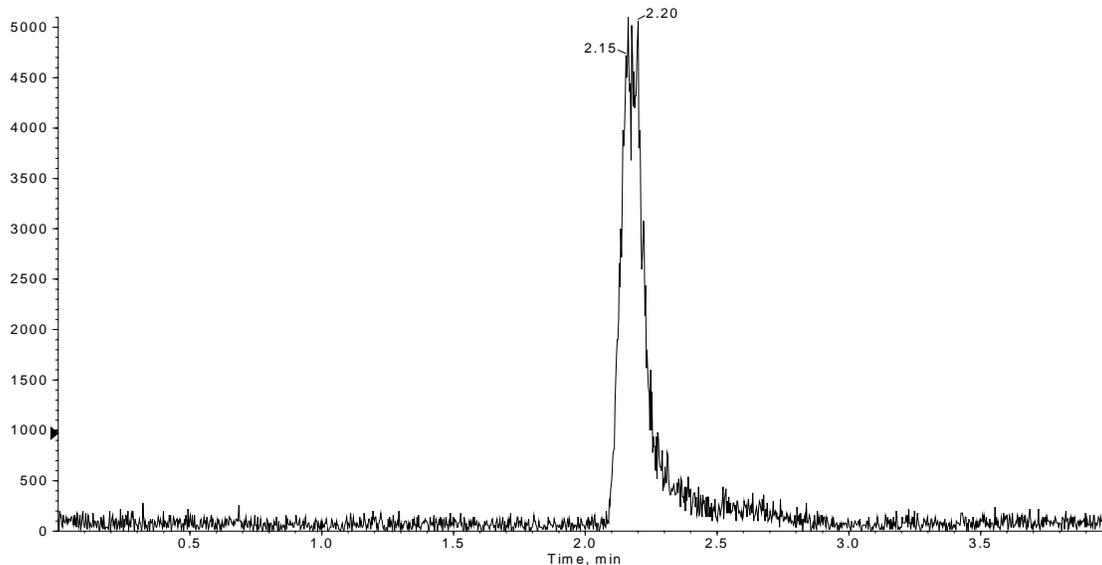


Figure D.4: Internal standard tri-deuterium nicotine in the sample of figure D.3.

D.1.2 Teflon TF-1000 filters

In the NRC analysis of nicotine extracted of the Teflon filters and the NRC, the limit of detection for the concentration of nicotine is estimated to 10 ng/ml.

D.1.2.1 NRC in air when no irritation is noticed

The analysis of the Teflon filters from the sampling in the Nicorette gum production gave the results shown in the figures below. Figure D.5 shows the chromatogram of nicotine in the air when no irritation occurs, below the irritation level. Figure D.6 shows the chromatogram of the internal standard tri-deuterium nicotine applied to the sample that is found in figure D.5.

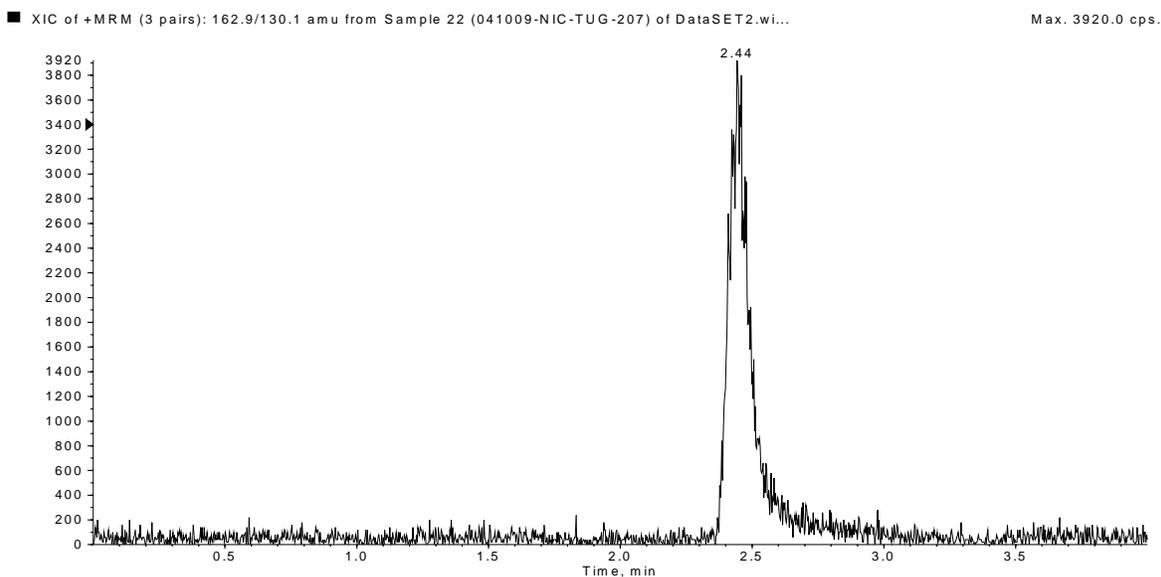


Figure D.5: Nicotine in the air when no irritation is noticed in the Nicorette gum production, Teflon filter.

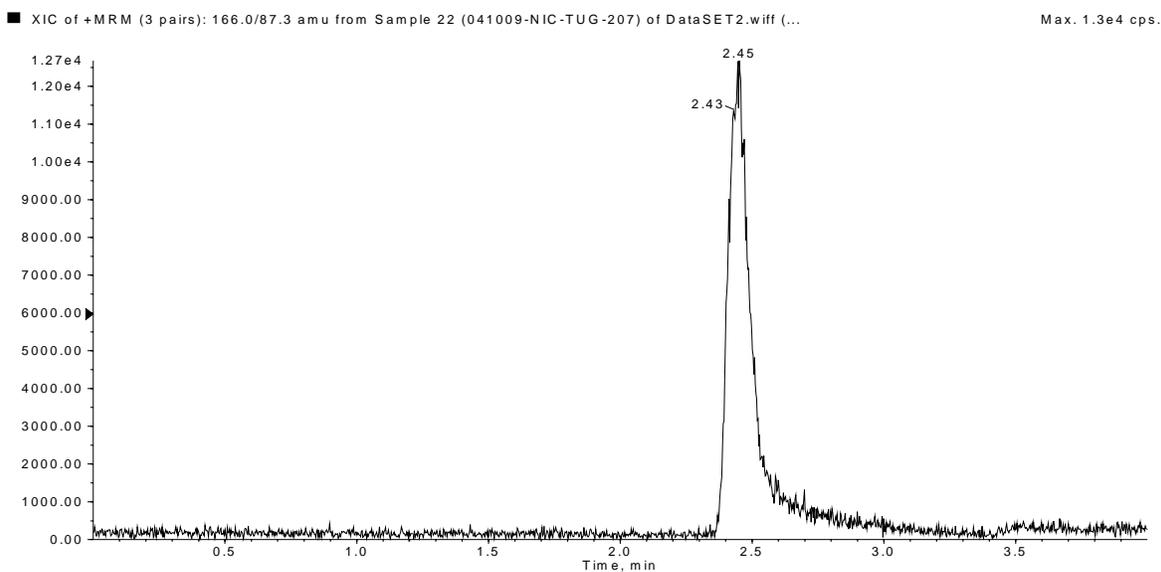


Figure D.6: Internal standard tri-deuterium nicotine in the sample of figure D.5.

D.1.2.2 NRC in air above irritation level

One result of the nicotine in the air, in the production room, when irritation occurs is shown in the chromatogram in figure D.7. Figure D.8 shows the chromatogram of the internal standard tri-deuterium nicotine that is applied to the sample of figure D.7.

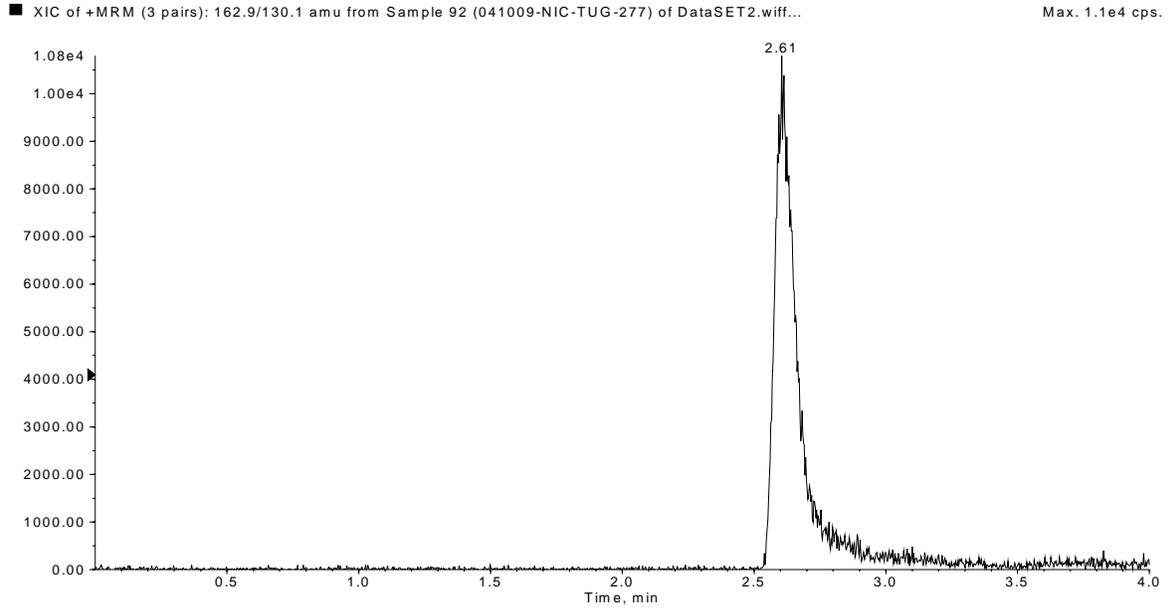


Figure D.7: Nicotine in the air above irritation level in the Nicorette gum production, Teflon filter.

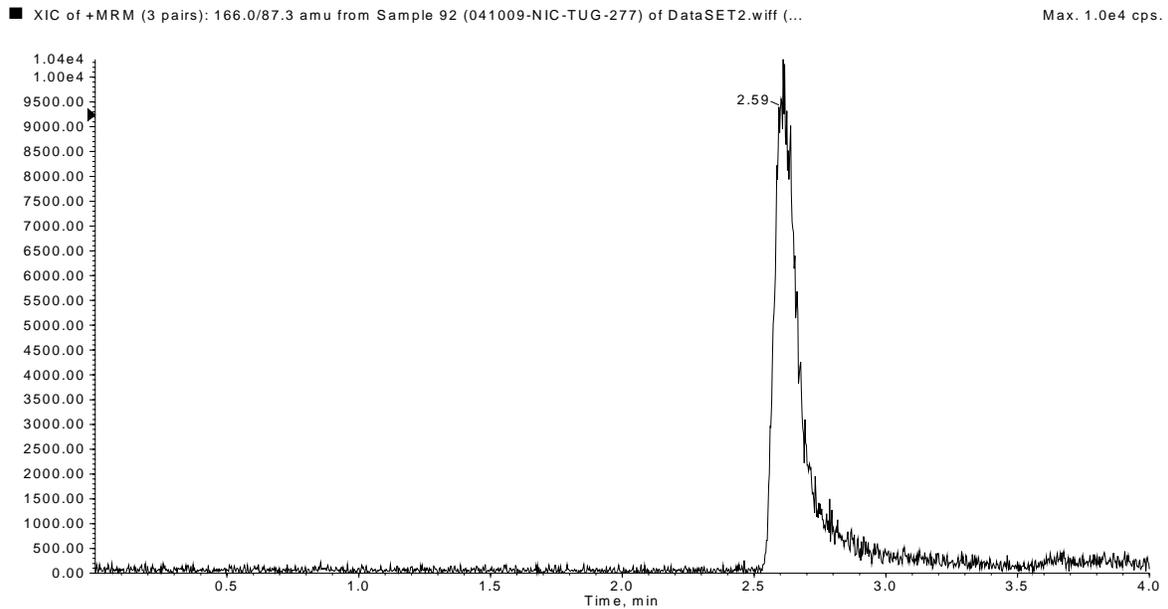


Figure D.8: Internal standard tri-deuterium nicotine in the sample of figure D.7.

D.2 Cotinine

For the biological samples of the specimens of urine, the estimated limit of detection for the concentration of cotinine is 1 ng/ml.

One peak of cotinine in the specimen of urine is shown in the chromatogram in figure D.9. The peak of the internal standard tri-deuterium cotinine from figure D.9 is shown in the chromatogram in figure D.10.

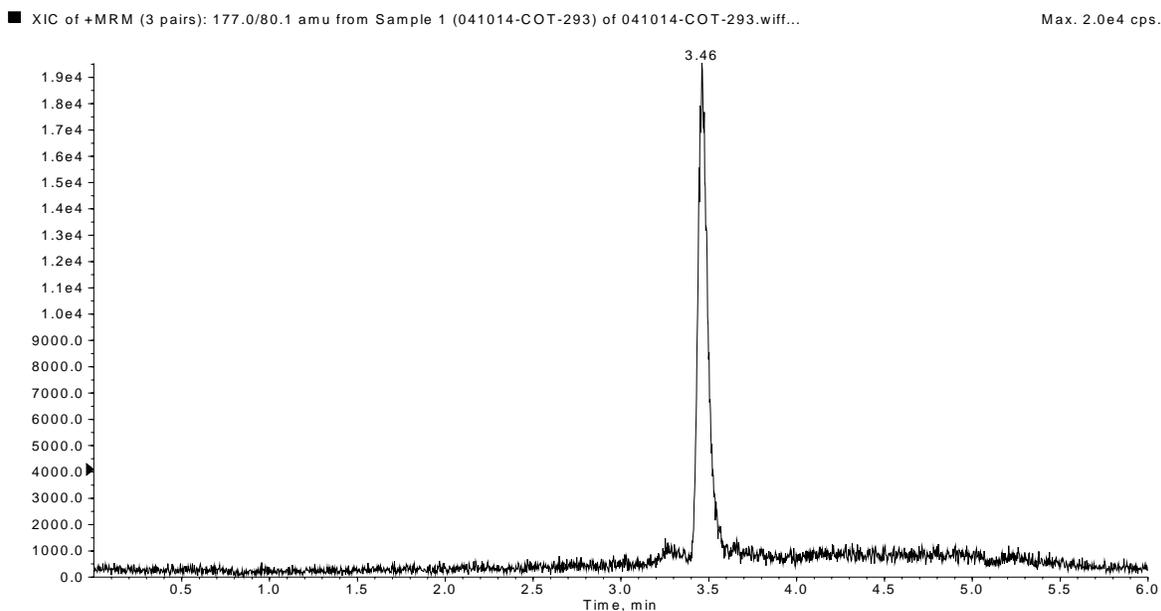


Figure D.9: Urinary cotinine from the exposure to nicotine in the Nicorette production.

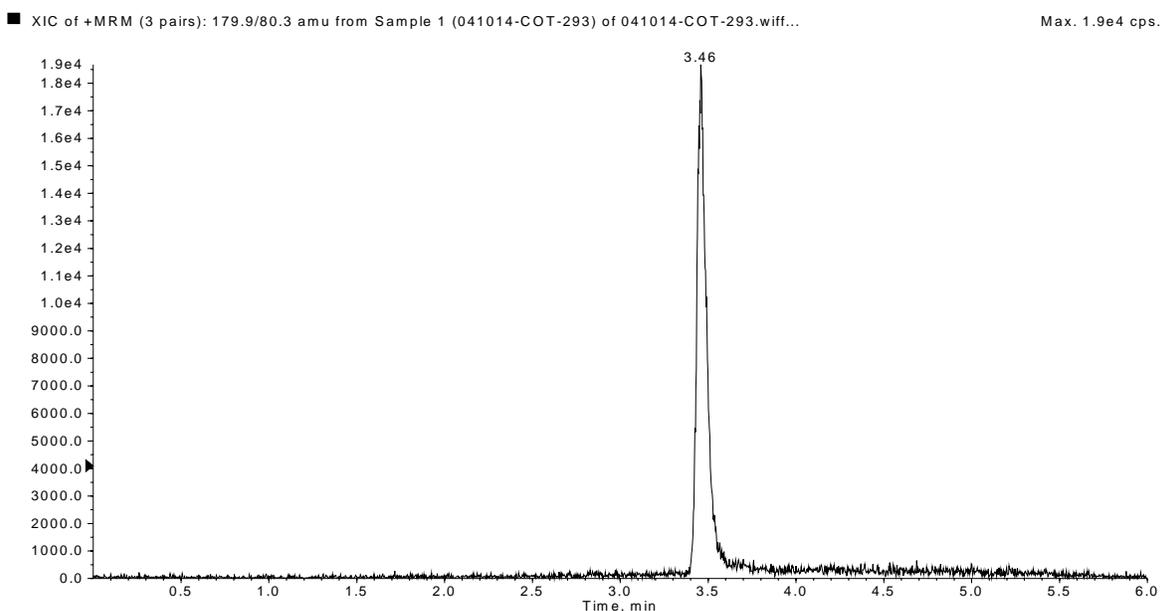


Figure D.10: Internal standard tri-deuterium nicotine in the sample of figure D.9.

D.3 Nicorette patch production

D.3.1 Concentration of nicotine in the air in the coating room

The results from the area sampling in the coating room are presented in figure D.11. The concentration of nicotine is low. Figure D.11 shows how much nicotine is present in the ambient air during a full working day, approximately eight hours. Sampling is done in the breathing zone in no direct connection with the Mark Andy (figure A.5.1 - Map of the coating room, Appendix 5). Days 9 and 17 have two result bars, as production was running in two coating rooms those days.

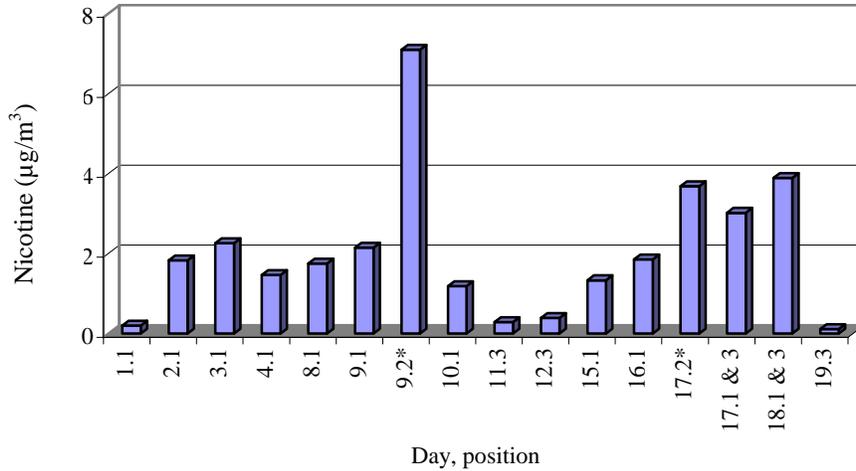


Figure D.11: Concentration of nicotine in the ambient air from stationary sampling, in the Nicorette patch production room. Every result bar shows an overall concentration of nicotine in the coating room for a working day. The pumps were positioned at different positions, 1, 2 and 3 in the room (figure A.5.1 Appendix 5) for air sampling. Day 9 and day 17 two coating rooms were in production see result with *.

Table D.1: Concentration of nicotine in the ambient air from stationary sampling, in the Nicorette patch production room.

	Median	Mean	Range
Nicotine $\mu\text{g}/\text{m}^3$	1.8	2.0	0.1 – 7.1

Days 11, 12 and 19 the pump with the absorbent XAD-2 tubes was further away from the Mark Andy than the other days. They are positioned close to the doors of the coating room (figure A.5.1, Appendix 5). The second bar, day 9*, and the first bar, day 17*, the XAD-2 tubes were positioned in coating room 2. The distance to the bench, where the Nicorette patches are stacked together, is shorter than in the coating rooms 1 or 3, where the other samples are taken. The first result bar day 17 and the result bar day 18 are measured both by the doors and close to the bench. Days 1 to 9 (first result bar), day 10 and days 15 to 16 the measurement was made at the same position by the wall where the garbage cans are (position 1, figure A.5.1, Appendix 5). The average concentration of nicotine vapor is only $2.0 \mu\text{g}/\text{m}^3$ (see table D.1), in the ambient air in the coating room, which lies very much below the TLV¹⁶ ($500 \mu\text{g}/\text{m}^3$). There is no connection between the

¹⁶ TLV = Threshold Limit Value

different concentrations of nicotine in the air in the coating room and the different Nicorette patch types: 5, 10 and 15 mg.

D.3.2 Concentration of nicotine in the air by the reception position

The concentration of nicotine in the air is much higher for the employees at the reception position (*figure A.5.1, Appendix 5*) than it is in the ambient air in the coating room. Figure D.12 shows the results from the nicotine analysis of the XAD-2 sorbent sample cartridges sampled at the reception position. During day 9 and day 17 measuring was done at the reception position in two coating rooms.

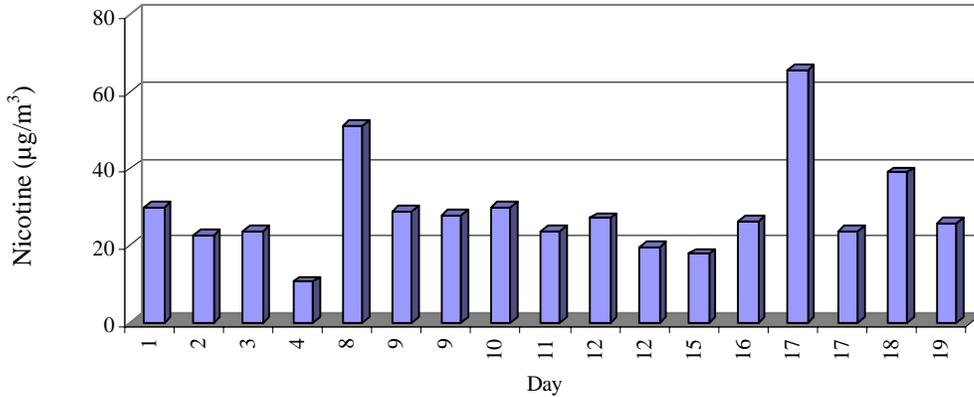


Figure D.12: Concentration of nicotine in the air by the reception position in the Nicorette patch production room. Day 12 two XAD-2 tubes were used at the same position. Production was going on in two rooms during days 9 and 17.

Days 3, 11 and 12 there were more than one XAD-2 tube measuring the concentration of nicotine by the reception position. The result bar of days 3, 11 and 12 in figure D.13 shows the concentration of nicotine while the Mark Andy runs one hour without any short stops¹⁷. It can be seen that more nicotine is present when the Mark Andy runs continuously. The result bar of day 11 (figure D.13) has the highest concentration result of all the measurements, 74.3 µg/m³. Because on that occasion the Mark Andy ran continuously.

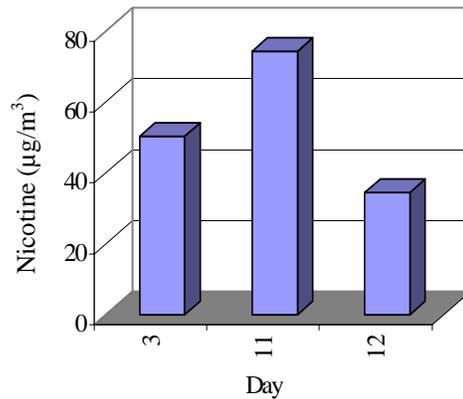


Figure D.13: Concentration of nicotine by the reception position by the Mark Andy during continuous production.

¹⁷ Short stop – the Mark Andy machinery is only stopped during the changing of the different articles of consumption when manufacturing.

Table D.2: Concentration of nicotine in the air by the reception position in the Nicorette patch production room.

	Median	Mean	Range
Nicotine $\mu\text{g}/\text{m}^3$	27	26	11 – 66
5 mg Nicorette patch $\mu\text{g}/\text{m}^3$	24	25	11 – 39
10 mg Nicorette patch $\mu\text{g}/\text{m}^3$	26	26	23 – 30
15 mg Nicorette patch $\mu\text{g}/\text{m}^3$	27	32	18 – 66
Nicotine (continuous production) $\mu\text{g}/\text{m}^3$	50	53	35 – 74

Table D.2 shows no significant patterns in the concentration of nicotine at this sampling position when different Nicorette patch types (5, 10 or 15 mg) are manufactured. Row number 5 in table D.2 shows the concentration of nicotine when the Mark Andy runs continuously.

D.3.3 Concentration of nicotine in the air by the waste roll at the Mark Andy

The sampling tools were attached to the Mark Andy (*figure A.5.1, Appendix 5*) to see how much nicotine the waste roll¹⁸ emits. The results are found in table D.3 and show the concentration of nicotine just near the waste roll. The results come from two different sampling days. After the waste roll is full, it is placed inside a plastic waste bag and thrown into a garbage can, placed inside the coating room. The handling of the full waste roll has not been measured separately. Table D.3 shows the median, the mean and the range of the concentrations of nicotine that is sampled.

Table D.3: Concentration of nicotine in the air by the Nicorette patch waste roll.

	Median	Mean	Range
Nicotine $\mu\text{g}/\text{m}^3$	4.8	4.8	4.8 – 4.8

D.3.4 Individual exposure in the coating room

In figure D.14 the individual exposure to nicotine is presented for one working day. When performing different working operations inside the coating room; the personal sampling equipment has been used during this time (*figure A.5.1, Appendix 5*). Table D.4 shows the median, the mean and the range from concentration of nicotine, the time spent in the coating room for all the employees participating in the study. Table D.4 also shows the median, the mean and the range of the total nicotine exposure and of the cotinine content in the specimens of urine from employees (marked with *) with a low zero value of cotinine on the first day of sampling.

¹⁸ Waste roll – it is the waste after the Nicorette patches are punched, which is rolled up.

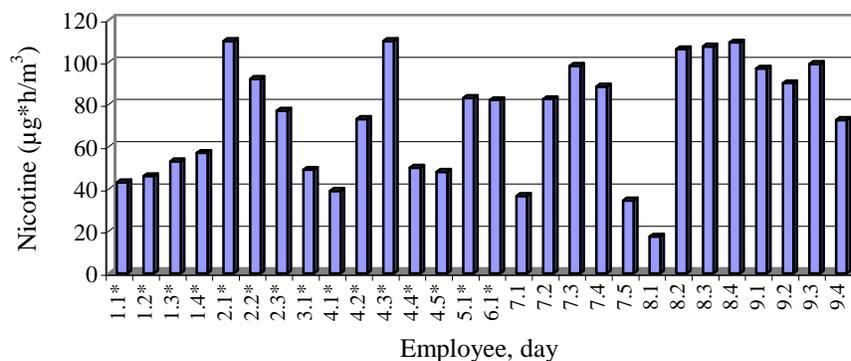


Figure D.14: Individual samples of the exposure to nicotine from the air by the Nicorette patch production. Every result bar shows the result of each of the concentration from the employee’s XAD-2 tube that particular day of sampling, multiplied by the time spent inside the coating room.

Table D.4: Individual samples of the concentration of nicotine from the air by the Nicorette patch production and the time the employees spent inside the coating room. The nicotine exposure and the values of cotinine, which are presented in the table (* in figure D.14 and table D.4) comes from the employees that had low zero values of cotinine in the specimens of urine on the first day of sampling.

	Median	Mean	Range
Nicotine concentration in the air $\mu\text{g}/\text{m}^3$	17	17	7.0 – 25
Time min	270	260	150 - 400
Nicotine exposure* $\mu\text{g}*\text{h}/\text{m}^3$	80	68	17 – 110
Cotinine* $\mu\text{mol}/\text{Creatinine mol}$	3.3	5.1	0.8 – 12
Cotinine* nmol/l	61	66	12 – 130
Cotinine* ng/ml	10	10	6 – 18

The results of the analysis of cotinine in the specimens of urine (samples with lower concentration of cotinine than 4 ng/ml), from the first day of exposure at the production, have been compared to the amount of nicotine in the XAD-2 tubes carried by the employees the first day, while they were working inside the coating room. The nicotine exposure values ($\mu\text{g}*\text{h}/\text{m}^3$) in table D.4 and figures D.14, D.15, D.16 and D.17 come from calculation 3, Appendix 6, which uses the nicotine exposure from the air found in the XAD-2 tube multiplied by the time in the production. Figure D.15 shows each person’s exposure to nicotine in the air and the following day’s contents of cotinine ($\mu\text{mol}/\text{l}$) in the morning urine divided by the contents of the creatinine (mol/l), see calculation 4b, Appendix 6. The nicotine exposure from the air and the urinary cotinine adjusted to the density of the urine, see calculation 4b, Appendix 6, are presented in figure D.16. Figure D.17 shows the concentration of cotinine from the specimens of urine, with no adjustments for any dilution or rate of secretion, and the exposure to nicotine in the air that working day.

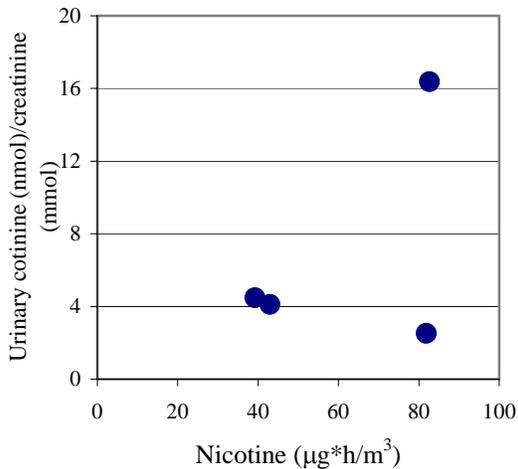


Figure D.15: Exposure to nicotine in the air, in the Nicorette patch production, and the urinary cotinine adjusted to the urinary creatinine, from the employees with a low zero value of cotinine.

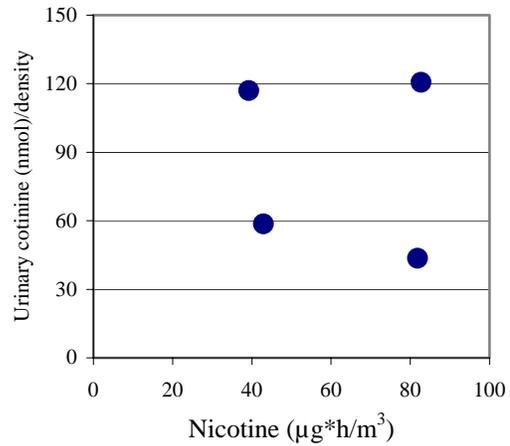


Figure D.16: Exposure to nicotine in the air, in the Nicorette patch production, and the urinary cotinine adjusted to the density of the urine, from the employees with a low zero value of cotinine.

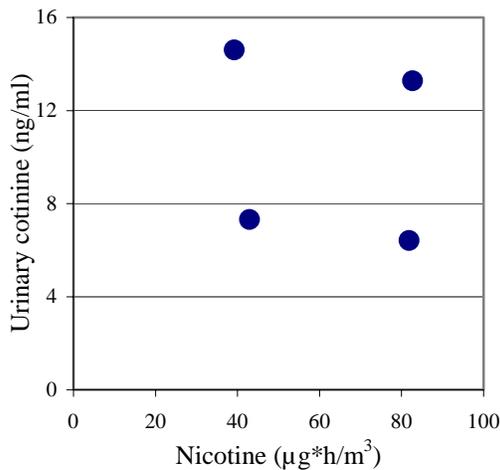


Figure D.17: Exposure to nicotine in the air, in the Nicorette patch production, and the concentration of cotinine in the specimens of urine, with no adjustments, from the employees with a low zero value of cotinine.

D.4 Nicorette gum production

D.4.1 Concentration of nicotine in the air in the mixing room

In figure D.18 the results of the concentration of nicotine in the air of the production room are shown. The results come from stationary area sampling (*figure A.5.2 - Map of the mixing room, Appendix 5*); they show the concentration of nicotine in the mixing room for one working day, approximately eight hours. Result bar day 2 shows a higher concentration of nicotine as that day the glove box was not closed when the NRC was filled into the container below, and on one occasion day 2 the air extraction through the perforated bench was not running either. Table D.5 shows the median, the mean and the range of the concentrations of nicotine found in the samples from the mixing room for the Nicorette gum.

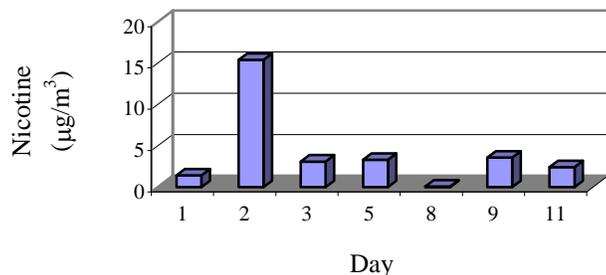


Figure D.18: Concentration of nicotine from the NRC in the air in the Nicorette gum production room. The results come from stationary sampling and show the average concentration of nicotine per working day.

Table D.5: The average concentration of nicotine (NRC) in the air per day from stationary sampling in the Nicorette gum production room.

	Median	Mean	Range
Nicotine (NRC) $\mu\text{g}/\text{m}^3$	3.1 (15)	4.2 (21)	0.1 – 15 (0.5 – 75)

D.4.2 Individual exposure in the mixing room

Figure D.19 shows the individual exposure to the nicotine in the NRC found in the personal air samples from the Nicorette gum production, multiplied by the time spent inside the mixing room, (figure A.5.2, Appendix 5). Table D.6 shows the median, the mean and the range of the concentration of nicotine and NRC, the time the employees were in the mixing room, the total nicotine exposure from the NRC in the air and the values of cotinine, which are found in the specimens of urine.

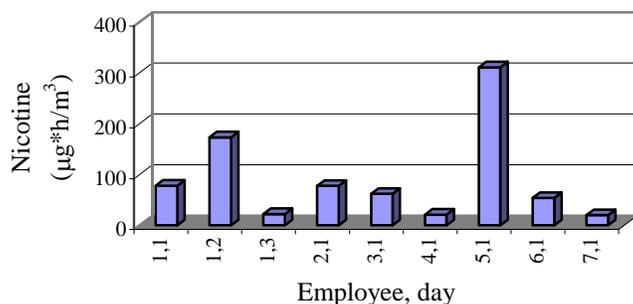


Figure D.19: Individual samples of the exposure to nicotine from the NRC in the air by the Nicorette gum production. Every result bar shows the result of each sample that particular day, multiplied by the time spent inside the mixing room.

Table D.6: Individual samples of the concentration of nicotine from the NRC in the air by the Nicorette gum production, the time the employees spent inside the mixing room, the total nicotine (NRC) exposure and the values of cotinine from the specimens of urine.

	Median	Mean	Range
Nicotine (NRC) $\mu\text{g}/\text{m}^3$	24 (120)	35 (175)	6.0 – 82 (30 – 410)
Time min	210	180	29 – 300
Nicotine (NRC) $\mu\text{g}\cdot\text{h}/\text{m}^3$	62 (310)	90 (450)	20 – 310 (100 - 1550)
Cotinine $\mu\text{mol}/\text{Creatinine mol}$	2.5	3.4	1.1 – 7.3
Cotinine nmol/l	42	61	8.7 – 130
Cotinine ng/ml	10	10	6 – 18

The urinary cotinine from the specimens of urine (samples with lower concentration of cotinine than 4 ng/ml), from the first day of exposure, to the nicotine measured in the airborne NRC that the employees were exposed to the first working day, is shown in figures D.20, D.21 and D.22. The exposure is estimated with calculation 3, *Appendix 6*. Figure D.20 presents the cotinine, adjusted to creatinine and the nicotine exposure that day, see calculation 4a, *Appendix 6*. Figure D.21 presents the cotinine, adjusted to the density of the urine and the nicotine exposure, see calculation 4b, *Appendix 6*. Figure D.22 shows the concentration of cotinine from the specimens of urine with no adjustments for any dilution or rate of secretion and the exposure to nicotine from the NRC in the air that working day.

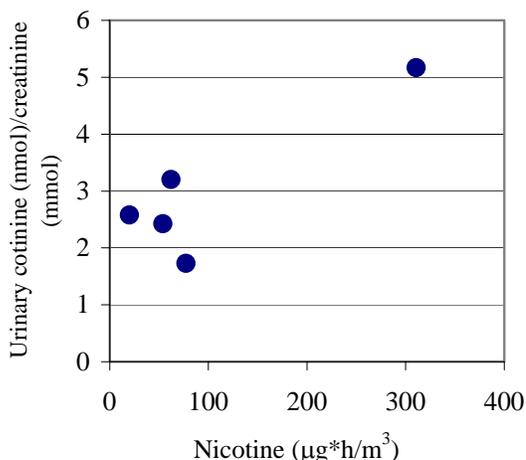


Figure D.20: Exposure to nicotine from the NRC in the air, in the Nicorette gum production, and the urinary cotinine adjusted to urinary creatinine.

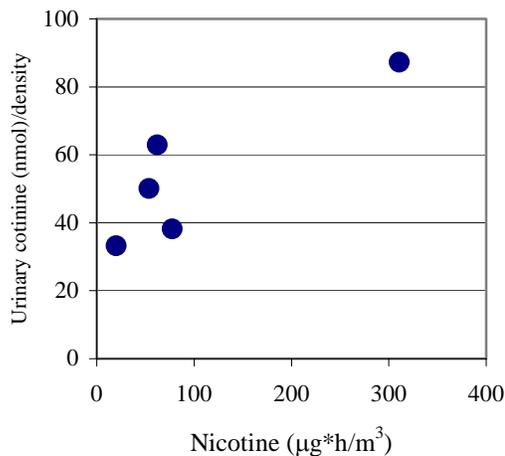


Figure D.21: Exposure to nicotine from the NRC in the air, in the Nicorette gum production, and the urinary cotinine adjusted to the density of the urine.

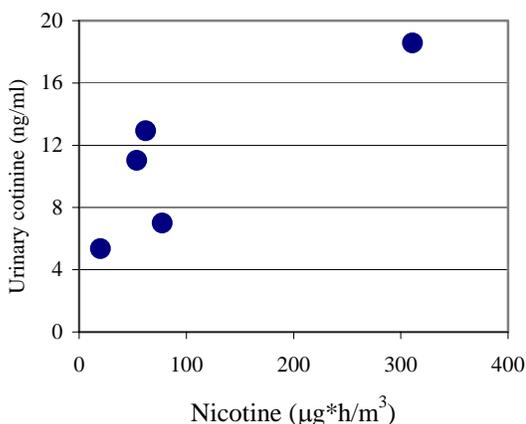


Figure D.22: Exposure to nicotine from the NRC in the air, in the Nicorette gum production, and the concentration of cotinine in the specimens of urine, with no adjustments.

D.4.3 Effects of the Nicotine Resin Complex 20% IRP

The samplings presented in D.4.3, for measuring the level of irritation of the NRC, have been done in the mixing room during very short sampling times (*see my position in figure*

A.5.2, Appendix 5). During days 1 to 5, filter 1,X to 5,X, 4 mg Nicorette gums were produced and days 8 to 12, filter 8,X to 12,X, 2 mg Nicorette gums. The results presented in figures D.23 and D.24, and tables D.7 and D.8 all come from samplings for between 1.5 and 5 minutes. The concentrations of nicotine ($\mu\text{g}/\text{m}^3$) shown in the figure below, figure D.23, caused no irritation. Table D.7, shows the median, the mean and the range of concentrations for nicotine from the NRC, when no irritation is noticed.

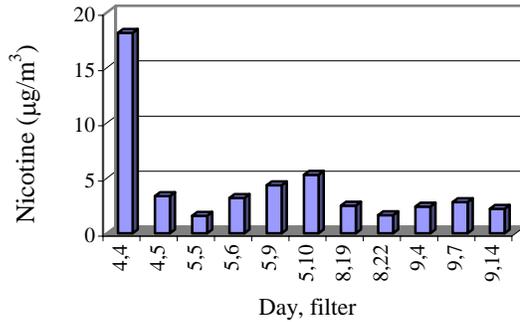


Figure D.23: Concentration of nicotine from the NRC in the air, in the Nicorette gum production room, when **no irritation** is noticed.

Table D.7: Concentration of nicotine (NRC) in the air, in the Nicorette gum production room, when **no irritation** is noticed.

	Median	Mean	Range
Nicotine (NRC) $\mu\text{g}/\text{m}^3$	2.8 (14)	4.3 (22)	1.6 – 18 (8.0 - 90)

Figure D.24, below, shows the concentration of nicotine ($\mu\text{g}/\text{m}^3$) from the NRC in the air when the irritation is noticed. The figure shows no specific difference between minor or severe irritation. Table D.8 gives the median, mean and range values for the concentrations of NRC and nicotine after irritation is noticed.

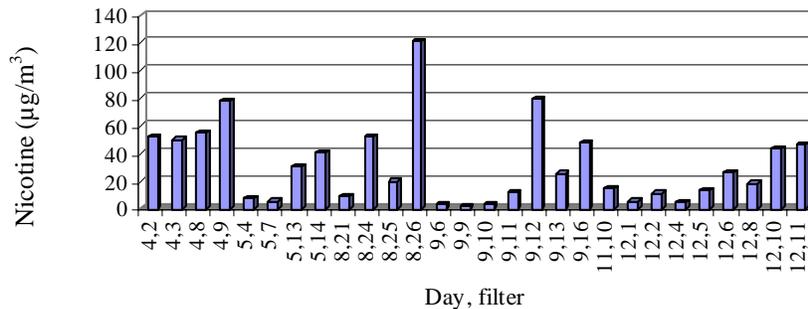


Figure D.24: Concentration of nicotine from the NRC in the air, in the Nicorette gum production room, when **irritation** is noticed.

Table D.8: Concentration of nicotine from the NRC in the air when **irritation** is noticed, in the Nicorette gum production room.

	Median	Mean	Range
Nicotine (NRC) $\mu\text{g}/\text{m}^3$	23 (115)	31 (155)	2.5 – 120 (13 – 600)

It is obvious that on some occasions no irritation was indicated, but on other occasions irritation occurred at lower and at the same levels of nicotine in the air.

E Discussion

The work at Pfizer's Nicorette production involves constant exposure to nicotine. The concentration of nicotine in the manufacturing rooms is many times and sometimes more than several hundred times lower than the TLV. The amount of nicotine in the air varies rather much between different areas in the rooms. Consequently the exposure varies depending on the different tasks that are performed.

E.1 Concentration of nicotine in the air in the Nicorette patch and Nicorette the gum production rooms

In the Nicorette patch production room, the coating room, the mean concentration of nicotine in the ambient air is $2 \mu\text{g}/\text{m}^3$ (table D.1). In figure D.11 clear differences can be seen between the different sampling places where the XAD-2 tubes with pumps were stationed. The closer to the bench, where the Nicorette patches were kept until stored, the sampling was done, the higher was the concentration of nicotine. The concentration in the coating room is on average more than 10 times lower than the average concentration of nicotine at the reception position of the Nicorette patches, $26 \mu\text{g}/\text{m}^3$, (table D.2). In table D.3, the concentration of nicotine by the patch waste roll is presented. It was measured to less than $5 \mu\text{g}/\text{m}^3$, which indicates that well positioned point air extractors reduce the concentration of nicotine in the air close to the Mark Andy.

In the Nicorette gum production room, the mixing room, the concentration of nicotine in the air from the NRC is found to be $4.2 \mu\text{g}/\text{m}^3$, which is equal to $21 \mu\text{g}/\text{m}^3$ of NRC on an average working day, table D.5 and figure D.18. That is twice as high a concentration of nicotine as the concentration in the Nicorette patch production. In this production, the source of NRC in the air is limited to the tasks when the NRC is filled into the containers. Then there is a high, temporary rise of NRC in the air.

The concentration of nicotine in the Nicorette patch and the Nicorette gum production rooms are below the TLV stated to $500 \mu\text{g}/\text{m}^3$.

E.2 Exposure to nicotine from the air in the Nicorette patch and the Nicorette gum production

The concentrations of nicotine in the individuals at the Nicorette patch production (figure D.14) were several times higher than the concentration in the ambient air. The employees are exposed to an average concentration of $15 \mu\text{g}/\text{m}^3$ nicotine from the air, for a working day. They work inside the coating room for 4 hours and 25 minutes on average. During this time they are at the reception position, where most of the exposure comes from, for 2 to 3 hours per day. They take the Nicorette patches from the reception to the bench where they are for at least another hour and throw away the waste roll or change the nicotine bottle for 15 to 30 minutes, but some days they never perform those tasks. The individual air sample of nicotine may have a higher concentration when the employee has changed the nicotine bottle, though a mask with a fresh airflow is used. The pump with the XAD-2 is placed offside the mask and nicotine vapor may therefore reach the XAD-2 cartridge

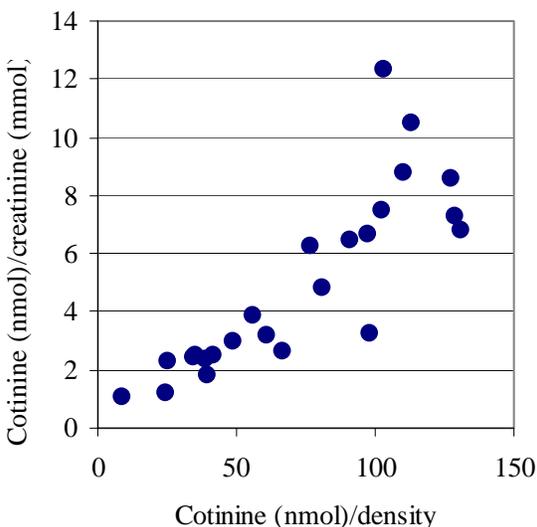
but not the employee. The individual difference of the nicotine exposure from the air is mostly due to the difference in time, spent at the reception position. The exposure (calculation described in chapter C.3) in the production of Nicorette patch is on average $69 \mu\text{g}^*\text{h}/\text{m}^3$ from the nicotine in the air (table D.4).

The employees, in the Nicorette gum production, do not stay inside the mixing room for an entire shift, on average they work there for 2 hours and 55 minutes (table D.6). There they are exposed to a greater concentration of nicotine, from the NRC, than the average concentration of nicotine vapor in the coating room, see tables D.4 and D.6. That is because they handle the NRC, which dusts. In table D.6 the individual nicotine exposure from the NRC is found to be on average $90 \mu\text{g}^*\text{h}/\text{m}^3$, which equals $450 \mu\text{g}^*\text{h}/\text{m}^3$ of NRC, from the calculation described in chapter C.3.

Depending on the two different Nicorette gum types, 2 and 4 mg, it can be seen that a greater concentration of NRC is found in the air when the 4 mg gums are produced. When the double quantity of NRC is filled into the container more NRC-dust fills the room, see figure D.23, D.24 and D.25.

E.3 Exposure to nicotine correlated to the concentration of cotinine in the specimens of urine

The cotinine is present at a very low concentration in mostly all humans. The exposure from environmental tobacco smoke is one source of the urinary concentration of cotinine, another source can be food that contains nicotine. Even the specimen of urine, which was used to the standard series, had a small concentration of cotinine. To correct the concentrations of cotinine in the calibration standard series, 1.1 ng/ml cotinine was added



to all the concentrations which were used to the calibration standard series. Urinary cotinine in the employees' samples was fitted to the different standard curves. One standard curve was used for results with concentrations below 10 ng/ml (57 nmol/l) and another for results with concentrations above. For the curve with low concentrations of urinary cotinine, the points of the highest concentrations of the standard series were not used.

The relation between the concentrations of cotinine adjusted to the two parameters, urine density and urinary creatinine, is shown in figure E.1.

Figure E.1: Concentration of cotinine adjusted to urine density compared to cotinine adjusted to the concentration of creatinine.

The concentration of cotinine adjusted either to urinary density or concentration of creatinine has clear individual differences. The creatinine adjustment is very individual as well as the adjustment to density. [10] The individual metabolic differences are also very important. Some people metabolize more cotinine than others and consequently the results between different people can vary a lot. [2, 3]

The values of exposure to nicotine from the air and the corresponding concentrations of cotinine from another study [13] were used to determine if there was another source of exposure than from the air. In figure E.Y, the exposure to nicotine and the concentration of cotinine from the employees in the Nicorette patch production is compared to the subjects' (from [13]) exposure to nicotine, presumably from the air, and their concentration of cotinine. The same kind of comparison between the Nicorette gum employees' exposure to nicotine from the NRC in the air and the concentration of cotinine, and the other study's [13] nicotine and cotinine values has been done. In the figures E.2 and E.3, the zero concentrations of cotinine in the urine were low, below 4 ng/ml.

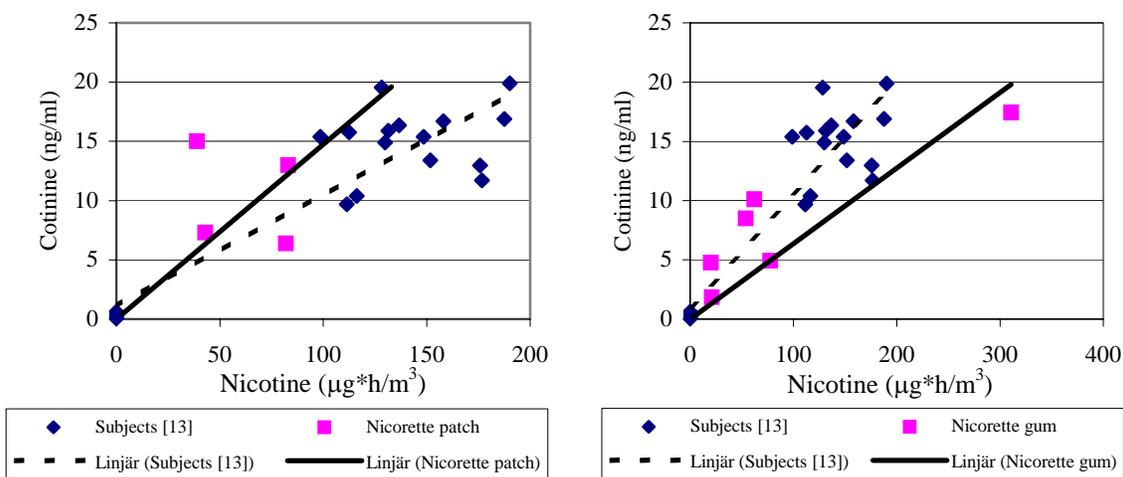


Figure E.1: Linear regression of the exposure to nicotine in the air at the Nicorette patch production and the concentration of cotinine in the specimens of urine. The dotted regression line corresponds to urinary cotinine and a presumable exposure to nicotine from the air [13].

Figure E.2: Linear regression of the exposure to nicotine from the NRC in the air at the Nicorette gum production and the concentration of cotinine in the specimens of urine. The dotted regression line corresponds to urinary cotinine and a presumable exposure to nicotine from the air [13].

The exposure to nicotine in 2004 at the Nicorette patch and the Nicorette gum production, compared to the exposure in the year 2002 at the same production sites is shown in the tables below. Tables E.1.1 and E.1.2 present the Nicorette patch production and tables E.2.1 and E.2.2 present the exposure at the Nicorette gum production.

Table E.1.1: Median, mean and the range of the exposure to nicotine in the air in the Nicorette patch production, urinary concentration of cotinine, cotinine adjusted to creatinine and cotinine adjusted to density.

2004	Median	Mean	Range
Nicotine $\mu\text{g}^*\text{h}/\text{m}^3$	80	68	17 – 110
Cotinine ng/ml	10	10	6 – 18
Cotinine $\mu\text{mol}/\text{Creatinine mol}$	3	5	1 – 12
Cotinine nmol/l	61	66	12 – 130

Table E.1.2: A mean value of a calculated exposure to nicotine in the Nicorette patch production in 2002, median, mean and the range of the urinary concentration of cotinine and cotinine adjusted to creatinine.

2002 [14]	Median	Mean	Range
Calculated value of exposure to nicotine $\mu\text{g}^*\text{h}/\text{m}^3$	-	100	-
Cotinine ng/ml	6	11	1 – 49
Cotinine $\mu\text{mol}/\text{Creatinine mol}$	4	6	2 – 22

In the Nicorette patch production, for three persons, there must be some other source of exposure than the exposure from the air, according to figure E.1. In tables E.1.1 and E.1.2 it can be seen that the levels of cotinine in the urine are approximately the same, while the calculated value of exposure to nicotine in 2002 is around 50 % higher than the exposure to nicotine that has been measured in the air samples. The urinary cotinine shows that the exposure to nicotine overall in the Nicorette patch production has not been reduced since the study of the exposure to nicotine in 2002.

Individually, it is clear that some employees (1, 4 and 5 in *table A.1.1, Appendix I*) must have been exposed to more nicotine than the nicotine vapor present in the air. It is their points in figure E.1 that increase the gradient of the regression line for the concentration of cotinine to the exposure of the nicotine plot. The amount of cotinine in the specimens of urine from the employees at the Nicorette patch production does not show a direct relationship between the nicotine in the air and the concentration of cotinine (ng/ml). The same can be said about figures D.15 and D.16. On average the urinary cotinine increases without any direct increase of the concentration of nicotine in the air. That extra urinary cotinine must come from another source of exposure. That source is probably the dermal route. Nicotine enters the skin very easily. Sometimes the employees use one pair of rubber gloves on top of cotton textile gloves (employees 1, 3 and 5, *table A.1.1, Appendix I*), which do not protect the hands from exposure as much and for as long as two layers of the rubber gloves do [11]. The employees may have used the same pair of rubber gloves for too long as well, and thus the nicotine might have penetrated the rubber and reached the skin.

In the Nicorette gum production the employees use their rubber gloves for just the moments they handle the NRC, filling the containers. They also normally wear a

disposable mask for protecting the respiratory tract from exposure to NRC. Nicotine is only released from NRC in contact with saliva, water or when heated [15].

The exposure to nicotine, from the NRC, in the Nicorette gum production has been reduced since 2002 according to the cotinine content found in the employees' urine, see tables E.2.1 and E.2.2.

Table E.2.1: Median, mean and the range of the exposure to nicotine, from the NRC, in the air in the Nicorette gum production, urinary concentration of cotinine, cotinine adjusted to creatinine and cotinine adjusted to density.

2004	Median	Mean	Range
Nicotine (NRC) $\mu\text{g}^*\text{h}/\text{m}^3$	62 (310)	90 (450)	20 – 310 (100 – 1550)
Cotinine ng/ml	9	11	2 – 23
Cotinine $\mu\text{mol}/\text{Creatinine mol}$	3	3	1 – 7
Cotinine nmol/l	42	61	9 – 130

Table E.2.2: Range values of a calculated exposure to nicotine from the NRC in the Nicorette gum production in 2002, median, mean and the range of the urinary concentration of cotinine and cotinine adjusted to creatinine.

2002 [14]	Median	Mean	Range
Calculated value of exposure to nicotine (NRC) $\mu\text{g}^*\text{h}/\text{m}^3$	-	-	400 – 700 (2000 – 3500)
Cotinine ng/ml	37	37	11 – 62
Cotinine $\mu\text{mol}/\text{Creatinine mol}$	17	17	11 – 22

The employee correlated to result bar number 1, 2 and 3 in figure D.19 (employee with the numbers 1.1, 1.2 and 1.3 in *table A.1.2, Appendix 1*) did not use the glove box cupboard in the closed position when filling the container with NRC. Day 2 the extraction through the bench inside the glove box was not running, and the room was immediately filled with NRC-dust. The employee correlated to result bar 7, figure D.19 (employee number 5.1 in *table A.1.2, Appendix 1*), was using a half-mask when performing the tasks of handling the NRC and the door of the glove box was open. During the other time when he was working in the mixing room no mask was used at all. The result bar numbers 6 and 9 in figure D.19 (employees numbers 4.1 and 7.1 in *table A.1.2, Appendix 1*) shows the workers who, this particular day filled the NRC, into the container below the glove box on one occasion only. Number 6 (4.1) was in the mixing room for just half an hour on the day of sampling. The employee correlated to result bar number 4, figure D.19, (number 2.1 in *table A.1.2, Appendix 1*) worked during the longest time in the mixing room and performed four premixes according to all the instructions (it is possible during a working day, to perform five premixes when everything functions well). That result shows that if the directions are followed the exposure is not very high. The exposure is related to how much work is carried out on a particular day; the more work at the glove box cupboard – filling the container for NRC – the more exposure. The cleaning of the premix containers is often done with high-

pressure air, which makes the NRC at the container and wherever else it has settled, whirl up and the mixing room becomes dust-laden once more.

In figure E.3, it is clear that the concentration of cotinine is lower than the expected concentration of cotinine due to the nicotine exposure, for three persons, the one that used a half-mask¹⁹ and two that used the protective mask. The mask that protects the respiratory tract used by the employees when working inside the mixing room, in the Nicorette gum production, did according to the plot in figure E.3, keep the exposure to the NRC and also the nicotine low. For the other two persons in figure E.3, the exposure was not reduced. The risk of errors, see below and chapter A.3.2, is in fact too big to say for sure that the mask protects the respiratory region from exposure to the NRC.

The metabolic differences cannot be neglected either, as mentioned earlier (chapter A.3.2). When the urinary concentrations of cotinine are as low as they are in this research, the exposure from other sources, like the environmental tobacco smoke, may have a big influence on the analytical results. Exposure from other sources may give a concentration of cotinine in the specimens of urine that does not correspond to the air samples of the concentrations of nicotine when they are as low as they are in the coating and the mixing rooms.

All the results from the specimens of urine cannot be used as some subjects have been exposed to other sources of nicotine. One employee had a zero-concentration of cotinine several hundred times higher than the non-exposed employees (see employee number 9, table A.1.1). These amounts of cotinine have a nicotine source corresponding to several cigarettes. A couple of other employees (see employees numbers 7 and 8, table A.1.1) must also have been exposed to much environmental tobacco smoke, or used nicotine days prior to the sampling of urine, so their urinary cotinine was not used either.

E.4 Effects from the Nicotine Resin Complex 20% IRP in the air

The symptoms of irritation that have been studied are my own. Consequently, the level of sensation is subjective and thus the results. Notes of how much irritation occurred and if the employees felt irritation as well, were done while the air samples were collected. The irritation from the NRC that I felt appeared especially in the nasal cavity, the pharynx and the larynx regions and consisted of coughs and a sour throat. I could notice that I got accustomed to and insensible to the irritating NRC dust when being in the mixing room during a working day. The longer the time I spent inside the mixing room, when the tasks of filling the containers with the NRC were performed, the less acute were the irritation and the severe coughs. But even when not coughing I was always feeling the sour throat. It was obvious that whenever the employees coughed I coughed as well, but often I coughed and the employees did not. For the day-to-day level of irritation I could feel less severe irritation but no indications of any trend of absence of irritation from the NRC dust. I could feel the sour throat for days after being exposed to the NRC.

¹⁹ Half-mask – a semi-protective mask, which has a filter for particles and covers the mouth and nose.

When NRC dust is inhaled it gives irritations in the upper respiratory tract, as 70-90% of the particles, which are larger than 10 μm in aerodynamic diameter, are deposited before the inhaled air reaches the larynx. [8] The employees in the production room are exposed to the irritating NRC and the other dusty compounds, which the Nicorette gum consists of. In the study of the irritation of the NRC, the results from *when irritation was noticed*, with sampling times lower than 4 minutes were studied extra carefully, it can be seen that there is a tendency that the tolerance to the NRC dust has increased during the day. For the day-to-day level of irritation, no trend could be detected whether the level was raised or not. When irritation from the NRC starts to be noticed, the concentration of nicotine from the air samples has been measured to a range of 2.5 to 120 $\mu\text{g}/\text{m}^3$, which is equal to a range of NRC of 13 to 600 $\mu\text{g}/\text{m}^3$.

The symptoms of irritation start when the NRC is handled. The other Nicorette gum ingredients that dusts do not give irritations when they are handled separately from the NRC.

The NRC samplings, whose results are presented in chapter D.4.3, have been done with very different sampling times (chapter D.4.3). Therefore there are risks of errors in the results. The short times of sampling have less chance to show the right concentration in the air of the measured substance. In this study just a very small volume of the filtered air containing NRC, from the mixing room, was sampled and analyzed.

E.5 Methods

The recovery of the method extracting the nicotine from the Teflon filters to ammonia hydroxide and the following liquid-to-liquid extraction to ethyl acetate was not calculated. The results in the Nicorette gum part must therefore be studied with criticism.

The limits of detection for the analytical series were not established. Instead they were estimated, due to the calibration standard series for each of the analytical series. The limit of detection at 10 ng/ml for nicotine vapor, gives a possibility to measure concentrations of nicotine in the air at 1 $\mu\text{g}/\text{m}^3$ when sampling for 10 minutes at a sampling rate of 2 liters per minute. For four hours of sampling at a sampling rate of 1 liter per minute, concentrations of nicotine vapor in the air that can be detected can be as low as 0.1 $\mu\text{g}/\text{m}^3$. For the NRC the limit of detection is estimated to 10 ng/ml as well. Concentrations of nicotine from the NRC that are sampled in the air at a rate of 2 liters per minute can be detected at 0.8 $\mu\text{g}/\text{m}^3$, which equals 4 $\mu\text{g}/\text{m}^3$ of NRC. For four hours of sampling, 0.1 $\mu\text{g}/\text{m}^3$ of nicotine extracted from the NRC sampled in the air is detectable, a concentration of nicotine that equals 0.5 $\mu\text{g}/\text{m}^3$ of NRC.

Analyses of nicotine from the air samples taken in the Nicorette production were run and carry over effects could be observed when samples with a large concentration of nicotine were found. Carry over effects could be seen when samples with very low concentrations were run directly after those with large concentrations. Consequently it was difficult, sometimes impossible, to evaluate the right concentration in the sample with a low concentration. The carry over effects were between one and two percent of the

concentrations of the samples. To examine the magnitude of the carry over effect, samples with a high concentration of nicotine were run with more than one blank afterwards. The nicotine-containing samples gave the carry over effect from the nicotine, mentioned above, in the blank samples.

In the Nicorette patch sample analysis, the first layer (A) was run prior to the second layer (B). Generally layer A contained a high concentration of nicotine. Layer B had less concentration, around 5 percent of that of A. After layer B had been analyzed, the process was repeated, starting with layer A from another XAD-2 tube. The carry over effect from layer A was not excluded in the calculation of the contents of nicotine in the XAD-2 tube. Carry over effects from layer B that were analyzed in the run before were negligible to the concentration of nicotine in the second sample of layer A.

In the air samples from the Nicorette gum production concentrations of nicotine were very low in some samples. First all samples from the Teflon filters were run one after the other. Carry over effects did definitely influence the results when the concentrations were low. Therefore the samples with low concentration were run again with blanks (only the organic mobile phase - methanol) in between. The concentrations of the samples were then correctly analyzed.

F Conclusions

According to the comparison between the exposure to nicotine from the air in the Nicorette patch production and the concentration of cotinine, in a few cases the cotinine content in the urine was higher than the potential exposure to the nicotine vapor in the surrounding air. That extra exposure might come from more than one source of exposure, probably from when the patches are handled, in combination with the negligence of not changing the gloves often enough. Or, when those tasks are performed using just one single pair of gloves instead of the double layer of gloves, which are supposed to be used.

In the Nicorette gum production, the employees' concentration of cotinine in the specimens of urine correlated to the exposure to nicotine from the NRC in the air shows a different relation than the one in the Nicorette patch production. The use of a protection mask may prevent the airborne NRC to reach the respiratory tract and the exposure to the NRC, and nicotine, is reduced.

In order to estimate the accurate irritation level of the NRC more studies must be done with more participants included. A Short Time Exposure Limit (STEL) of NRC in air must be considered to be established, that STEL should be $100 \mu\text{g}/\text{m}^3$. Until then a TLV for Nicotine Resin Complex 20% IRP is considered to be as low as $100 \mu\text{g}/\text{m}^3$.

The methods of extracting the nicotine from the Teflon filters and the liquid-to-liquid extraction must be validated before any final conclusions of the exposure to the NRC in the air can be affirmed.

The method of using the concentration of nicotine in the ambient air, in the Nicorette patch and the Nicorette gum production rooms, to calculate the total exposure is not reliable. The concentration of urinary cotinine varied very much in relation to the amount of nicotine that the employees were exposed to from the air in the coating room. In the Nicorette gum production there were also very large variations between the concentration of cotinine of the employees and their exposure to nicotine, from the NRC in the surrounding air.

To reduce the exposure in the Nicorette patch production even more, the design of the Mark Andy can be improved. More air extraction parts can be applied to the area where the Nicorette patches arrive at the reception position. At the reception, the patches could automatically be stacked together instead of making the two extra steps to collect them in stacks, by an employee and, move them over to the bench where they are wrapped in aluminum foil, by hand.

For the employees at the Nicorette gum production, the exposure to NRC (nicotine) has been reduced over the years. New solutions, like the glove box, where NRC is filled into the container, and the more airtight joint, between the premix container and the tube in the floor, have reduced the exposure of NRC (nicotine) at the Nicorette gum production. Even less exposure may be possible to achieve, if all the production directions would be followed. A more airtight glove box cupboard would reduce the exposure too.

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Abbreviations

amu	-	Atomic mass unit
HPLC / LC	-	High performance liquid chromatography
IS	-	Internal standard
m/z	-	Mass over charge
M	-	Molar (mol per liter)
MS	-	Mass spectrometry
MS/MS	-	Tandem mass spectrometry
NRC	-	Nicotine Resin Complex 20% IRP
rpm	-	Rotations per minute
STEL	-	Short Time Exposure Limit
TLV	-	Threshold Limit Value

Appendix 1 Exposure to nicotine and urinary cotinine

Table A.1.1: Exposure to nicotine from the air during the Nicorette patch production, urinary cotinine from the employees.

Employee and day of sampling	Concentration of nicotine in the air $\mu\text{g}/\text{m}^3$	Time <i>min</i>	Nicotine exposure $\mu\text{g}^*\text{h}/\text{m}^3$	Cotinine <i>ng/ml</i>	Cotinine μmol /Creatinine <i>mol</i>
1.0	-	-	-	0.4	0.3
1.1*	7.7	330	43	7.3	4.1
1.2*	10	260	46	10	6.7
1.3*	12	280	53	12	9.1
1.4*	13	270	57	14	11
2.0	-	-	-	6.1	2.0
2.1	23	280	110	14	5.3
2.2	20	270	92	13	6.2
2.3	20	230	77	13	4.7
3.0	-	-	-	14	3.3
3.1	15	190	49	21	7.4
4.0	-	-	-	3.9	1.5
4.1*	8.6	270	39	15	4.5
4.2*	14	320	73	22	11
4.3*	19	350	110	18	9.5
4.4*	11	270	50	17	8.7
4.5*	14	210	48	13	8.8
5.0	-	-	-	3.2	1.4
5.1*	25	200	83	13	16
6.0	-	-	-	0.4	0.3
6.1	17	280	82	6.4	2.5
7.0**	-	-	-	20	7.3
7.1	8.6	250	37	29	6.3
7.2	16	300	82	35	7.3
7.3	23	260	98	30	7.4
7.4	21	260	88	22	8.1
7.5	9.4	220	34	10	2.6
8.0**	-	-	-	290	190
8.1	7.0	150	17	110	37
8.2	21	320	106	180	54
8.3	23	280	107	95	33
8.4	24	270	109	52	20
9.0**	-	-	-	990	840
9.1	23	250	97	1100	1100
9.2	26	210	90	1100	1500
9.3	18	330	99	460	420
9.4	17	250	73	580	680

* The employee did not use a double layer of protection gloves during production.

** The employee's zero value of urinary cotinine is very high. The sampled specimens of urine the days after the exposure to nicotine in the production cannot be used.

Table A.1.2: Urinary cotinine from the employees in the Nicorette gum production, the exposure to nicotine from the NRC in the air and a value of nicotine exposure that can give the concentration of cotinine in the urine sampled.

Employee and day of sampling	Concentration of nicotine from NRC in the air $\mu\text{g}/\text{m}^3$	Time <i>min</i>	Nicotine exposure $\mu\text{g}^*\text{h}/\text{m}^3$	Cotinine <i>ng/ml</i>	Cotinine μmol /Creatinine <i>mol</i>
1.0	-	-	-	11	4.1
1.1*	17	270	78	28	12
1.2*	82	130	170	34	10
1.3*	24	55	22	30	9.7
2.0	-	-	-	2.1	0.9
2.1*	38	120	78	7.0	1.7
3.0	-	-	-	2.8	1.7
3.1*	13	300	62	13	3.2
4.0	-	-	-	5.4	2.8
4.1*	43	29	21	7.2	4.4
5.0	-	-	-	1.2	0.5
5.1**	76	250	310	19	5.2
6.0	-	-	-	2.5	0.5
6.1*	15	220	54	11	2.4
7.0	-	-	-	0.6	0.8
7.1*	5.8	210	20	5.4	2.6

* The employee used a mask while filling the NRC into the containers.

** The employee used a half-mask when filling the NRC, during the other part of the time inside the mixing room no mask was used at all.

Appendix 2 Material

2.1 Air sampling

Filter:	Gelman Sciences, Teflon TF 1000, diameter 37 mm, pore size 1 μm
Filter cassettes:	Omega Specialty Instruments Co., filter cassettes 37 mm
Plastic hose:	Latex tubing, Amber, VWR Scientific
Pump:	MSA, Escort ELF Pump, Aver
	SKC, Aircheck sampler model 224-52
XAD-2 tubes:	SKC Tube Xad-2

2.2 Sample extraction and preparation

Centrifuge:	Sigma 3E 1
Evaporator:	SPD 2010 Termo Savant
Refractometer:	Hand Refractometer, Ladassco ATAGO
Shaker:	Ika - Vibrax – VXR electronic

2.3 Chemicals

Ammonia solution 25%	Merck Eurolab AB
Ammonium acetate	Merck Eurolab AB
Cotinine,	Sigma Chemical Co.
D ₃ -cotinine	Sigma Chemical Co.
Ethyl acetate	Lab-Scan Analytical Sciences
Methanol	Lab-Scan Analytical Sciences
Nicotine	Janssen Chimica
D ₃ -Nicotine	Lab-Scan Analytical Sciences
Potassium hydroxide	Merck Eurolab AB
Water	H ₂ O-MilliQ at 18.2 M Ω

2.4 Analysis

LC-MS/MS:	Perkin-Elmer Biosystems, PE SCIEX API 3000, LC/MS/MS System Triple quadrupole LC-MS/MS mass spectrometer PE 200 Autosampler PE 200 Micropump
Software Version:	Analyst 1.4

Appendix 3 Chemical and physical data, classification and toxicological information

3.1 Chemical and physical data

3.1.1 Nicotine [5]

Synonym: 3-(2(N-methylpyrrolidinyl))pyridine

Molecular formula: $C_{10}H_{14}N_2$

CAS-number: 54-11-5

Molecular weight: 162.2 g/mol

Appearance: Oily, colorless hygroscopic liquid, turns brown on exposure to air. Sticky, characteristic odor.

Boiling point: 247°C

Melting point: -80°C

Density: 1.01 g/cm³

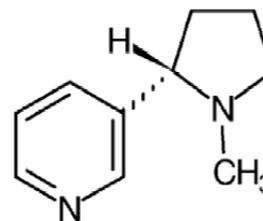
pK_{a1}: 7.84 at 25°C

pK_{a2}: 3.04 at 25°C

Octanol/water partition

coefficient: log P_{ow}: 1.2

Vapor pressure (20°C): 0.006 kPa



3.1.2 Nicotine Resin Complex 20% IRP [15]

Synonym: Nicotine Polacrilex

Molecular formula: Amberlite IRP64 Resin
(Divinylbenzene/methacrylic acid copolymer) (C₁₈O₄H₂₂)_n
Nicotine C₁₀H₁₄N₂

CAS-number: 96055-45-7

Appearance: White, dusty powder.

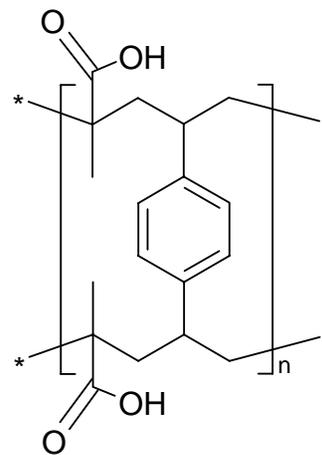
Boiling point: Not applicable

Melting point: No data available

Density: 0.4 g/cm³

Octanol/water partition

coefficient: log P_{ow}: No data available



3.2 Classification

3.2.1 Nicotine [5]

Classification: T+

Very toxic

N

Dangerous for the environment.

R-phrases:

R 26/27/28

Very toxic by inhalation, in contact with the skin or swallowed.

R 36/37

Irritating to eyes and respiratory system.

R 51/53

Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S-phrases:	S 36/37	Wear suitable protective clothing and gloves
	S 38	In case of insufficient ventilation, wear suitable respiratory equipment.
	S 45	In case of accident or if you feel unwell, seek medical advice immediately (show the label if possible).
	S 61	Avoid release of the substance to the environment. Refer to special instructions/Safety data sheets.
Exposure Limit Values:	ACGIH TLV (1992 – 1993): 0.5 mg/m ³ (TWA ²⁰) Skin	

3.2.2 Nicotine Resin Complex 20% IRP [5]

Principal Hazardous component:

Nicotine Resin Complex 20% IRP (NRC) is a compound of 20% nicotine in an ion-exchange resin. The nicotine will release in contact with perspiration or saliva.

Classification: Same as for nicotine.

R-phrases: Same as for nicotine.

S-phrases: Same as for nicotine.

Exposure Limit Values: Same as for nicotine.

3.3 Toxicological information

3.3.1 Nicotine [5]

LD₅₀ (oral rat): 50 mg/kg

Mutagenicity: Negative in Ames' test

3.3.2 Amberlite IRP64 Resin [16]

LD₅₀ (oral rat): >5000 mg/kg

Mutagenicity: Negative in Ames' test

Particle size:

5 %	> 710 µm
85 %	< 212 µm
100 %	> 1 µm [17]

²⁰ TWA –Time weighted Average for an 8 hours working day.

Appendix 4 Mass spectrometric settings

4.1 Nicotine

HPLC-column: Agilent Technologies, Zorbax Extend-C₁₈, 2.1 * 50 mm,
3.5 µm
Mobile phase: 10 mM ammonia acetate pH 9.5 (A), methanol (B)
Ionization: Electrospray, interface (setpoint) temperature 360°C

Injection details

Syringe Size: 250 µl
Injection Volume: 3 µl

Flush details

Pre-inject Flushes: 2
Post-inject Flushes: 2

Table A.4.1: Step table.

Step	Total time min	Flow rate µl/min	Gradient profile	A %	B %
0	0.0	500	1	99	1
1	1.0	500	1	99	1
2	1.1	500	1	5	95
3	2.2	500	1	5	95
4	2.3	500	1	99	1
5	4.0	500	1	99	1

Table A.4.2: Fragmentation table.

Q1 amu	Q3 amu	Dwell ms
162.9	130.1	50
162.9	84.3	50
166.0	87.3	50

4.2 Cotinine

HPLC-column: Agilent Technologies, Zorbax C18, 4.5 * 50 mm, 5 µm
Mobile phase: 10 mM ammonia acetate (A), methanol (B)
Ionization: Electrospray, interface (setpoint) temperature 450°C

Inject details

Syringe Size: 250 µl
Injection Volume: 5 µl

Flush details

Pre-inject Flushes: 1
Post-inject Flushes: 1

Table A.4.3: Step table.

Step	Total time min	Flow rate µl/min	Gradient profile	A %	B %
0	0.0	700	1	95	5
1	4.0	700	1	5	95
2	4.1	700	1	95	5
3	6.0	700	1	95	5

Table A.4.4: Fragmentation table.

Q1 amu	Q3 amu	Dwell ms
179.9	80.3	50
177.0	80.1	50
177.0	98.2	50

Appendix 5 Maps of Nicorette production rooms with sampling positions

5.1 Nicorette patch production room – the coating room

An overall ventilation runs all the time and three large air extraction regulators are installed there, one above the reception position, another one in the center of the room above the Mark Andy and a third one above where the liquid nicotine is.

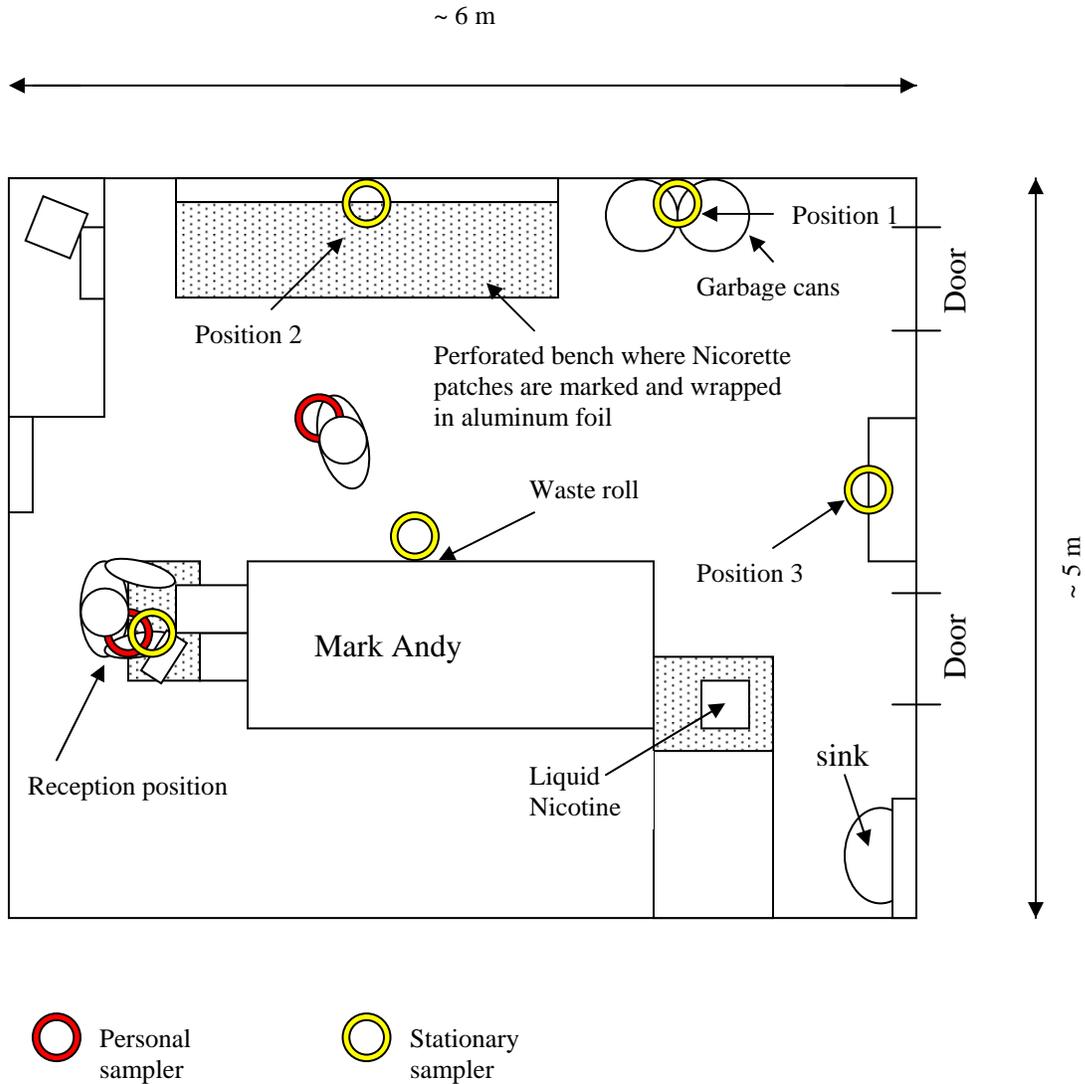


Figure A.5.1: Map of the coating room for the Nicorette patch production.

5.2 Nicorette gum production room – the mixing room

An overall ventilation runs all the time and two air extraction regulators are situated in the ceiling in the center of the room, one above the glove box and one above the cylinder in the right corner of the sketch.

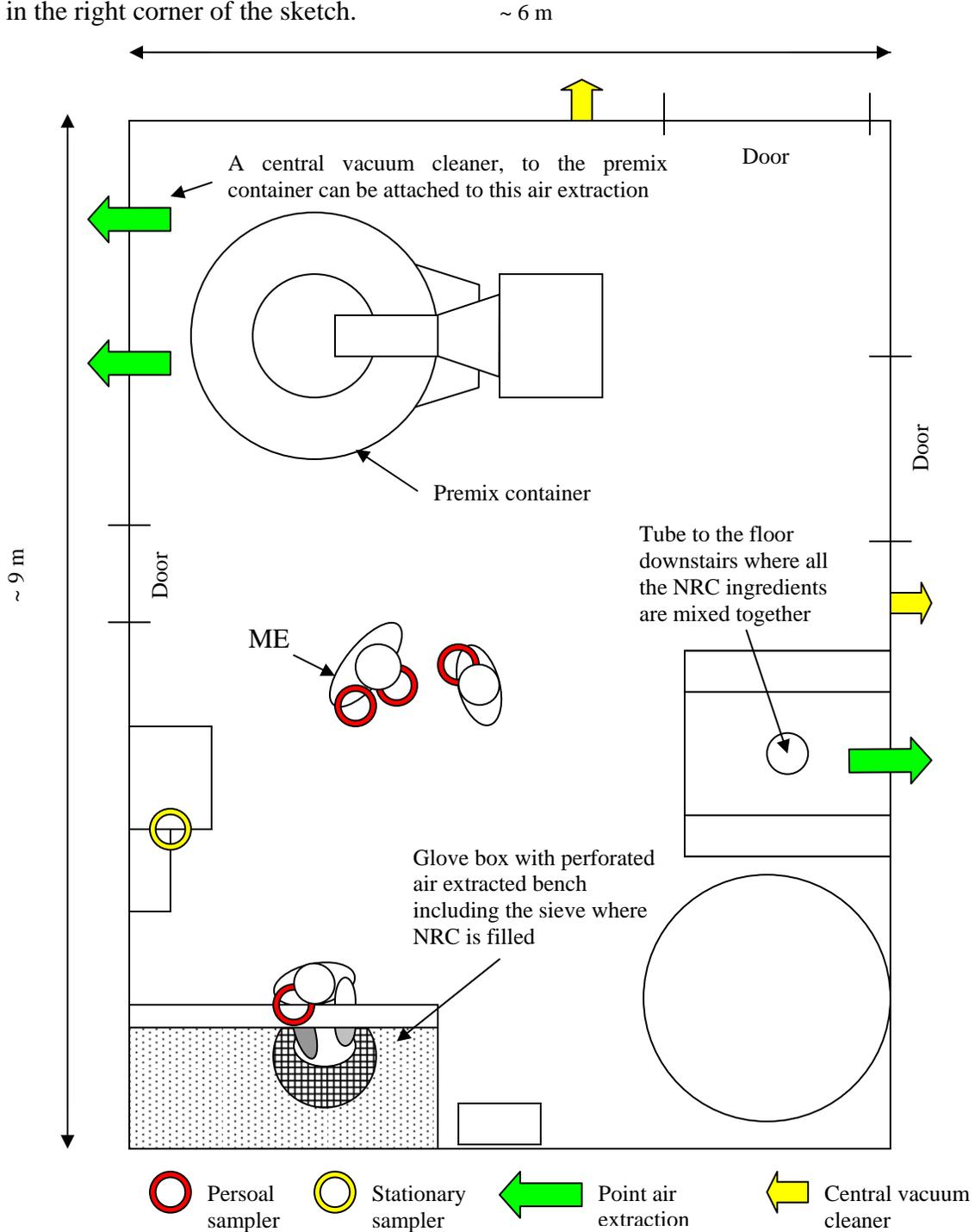


Figure A.5.2: Map of the Nicorette gum production room, the mixing room.

Appendix 6 Calculations

1 Concentration of nicotine in Nicorette patch production

$$2 \text{ [ml]} * ((A_{\text{layer}} + B_{\text{layer}})[\text{ng/ml}]) / (\text{sampled volume [m}^3] * 1000) = [\mu\text{g/m}^3]$$

2 Concentration of nicotine in Nicorette gum production

$$3[\text{ml}]/2 * (\text{filter content [ng/ml]}) / (\text{sampled volume [m}^3] * 1000) = [\mu\text{g/m}^3]$$

3 Exposure to nicotine in the Nicorette patch and Nicorette gum production

$$\text{Concentration of nicotine } [\mu\text{g/m}^3] * \text{Time for exposure [h]} = [\mu\text{g} * \text{h/m}^3]$$

4 Urinary concentration of cotinine

a) Correlated to creatinine:

$$\text{Concentration of cotinine [nmol]}/\text{concentration of creatinine [mmol]}$$

b) Correlated to urine density:

$$(\text{Concentration of cotinine [nmol/l]} * 0.024 \text{ [g/ml]}) / (\text{urine density [g/ml]} - 1) = [\text{nmol/l}]$$