POSTERS ABSTRACTS

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Medical University of Silesia in Katowice, Poland

Water pipe passive smokers’ exposure to nicotine

**Background:** Above 1.5 billion people are exposed to tobacco smoke every year. Since tobacco smoking prohibition was introduced in many countries, alarming trend of smoking alternative tobacco products is observed. One of aforementioned tobacco products is water pipe, which is nowadays very popular among youth. It seems to be reasonable to distinguish passive and active smokers’ exposure to water pipe smoke. Such smoke is probably as harmful as cigarette smoke.

**Aim of study:** The aim of this study was estimation of water pipe passive smokers’ exposure to nicotine.

**Materials and Methods:** 41 volunteers were classified as non-cigarette smokers group. They also declared tobacco abstinence since 30 days. 5 passive smokers from the non-cigarette smokers group were taking part in several research session (60 min exposure) alternatively. Urine samples were collected in session day and the day after. Nicotine, cotinine and trans 3'-hydroxycotinine concentration was estimated in collected urine samples using gas chromatography method with TSD detector.

**Results:** No statistically significant differences of analyzed biomarkers concentration was observed in samples acquired before and after 1 hour water pipe passive smoke exposure session. Results were compared with active water pipe smokers’ urine samples. In this case statistically significant differences were observed for cotinine (76.7±12.8 ng/ml vs. 329.1±62.5 ng/ml) and trans 3'-hydroxycotinine (271.2±92.7 ng/ml vs. 907.0±144.0 ng/ml). No statistically significant differences were found in case of nicotine.

**Conclusions:** Obtained results indicate no nicotine exposure during water pipe smoking on passive smokers. No statistically significant differences in nicotine and other metabolites concentration levels in urine samples were observed. Multiplicity of factors influencing passive exposure indicates need for further analysis e.g. concerning building ventilation and number of active smokers.
Electronic cigarettes - analysis of health warnings on packets

Significance: Electronic cigarettes called "e-cigarettes", are battery-powered devices that provide inhaled doses of nicotine. Many countries have already banned or strictly regulated e-cigarettes. However, they can be easily accessed online, also by children and adolescents. One controversy is whether electronic cigarettes can and should be regulated and labeled as drug-delivery devices or tobacco products.

Aim: Analyzed health warnings on packets and leaflets enclosed to electronic cigarettes and cartridges.

Materials and methods: 15 brands of e-cigarettes and 16 cartridges based on their popularity on national websites. All products were purchased from commercial sources and analyzed presence and content of the health warnings available on electronic cigarettes packets and information for customers.

Results: Analyzed packets of cartridges mainly was not direct information about nicotine dose in the product, storing condition and expiry date. Only few products had customer safety certificates. Although all examined products were purchased in Poland, some manuals were available only in English. Also found labels with information that e cigarettes are smoking cessation tools, they might be used in public places, where smoking is prohibited, they do not produce secondhand smoke, and are ecologically friendly. Some descriptions of unproved health benefits also were found in promotional materials. Moreover, some advertisements might encourage a non-smokers to try a new product.

Conclusions: E-cigarettes containing nicotine are not labeled enough. Electronic cigarette users have too little knowledge about nicotine dose, conditions of storing, and safely using this products.
Droplet size measurement of e-cigarette aerosols

Electronic Cigarettes are a new type of product rapidly gaining popularity with adult cigarette smokers. They typically produce a condensation aerosol by quickly evaporating a formulation containing nicotine and water with glycerol (VG), propylene glycol (PG) or a mixture of each. This study sought to measure aerosol droplet size distributions by real-time analytical methods using commercially available equipment. Measurements were conducted by electrical mobility (EM: Model DMS-500 MkII, Cambustion, UK) and by laser diffraction (LD: Spraytec, Malvern, UK). The Smoking Cycle Simulator (SCS: Cambustion, UK) was used to generate appropriate puff profiles, and to minimise dilution and potential droplet evaporation. Core profiles included 50, 55, 70 and 80 mL puffs of 3 s duration every 30 seconds. Volume-weighted median droplet diameters (d50) from a variety of e-cigarette devices were typically less than 500 nm by LD and less than 300 nm for EM, slightly larger than equivalent tobacco smoke measurements of approximately 210 nm. The electrical mobility data were larger than previous published data suggesting evaporation was in part suppressed. Precision data were dependent on the e-cigarette tested but coefficients of variation of less than 4–5% were observed for the better performing products. This degree of precision meets the acceptance criteria for droplet size distribution (d50 ± 20% for d50 < 1 µm) for laser diffraction measurements for similar aerosol products (British and European Pharmacopoeia Monograph XVII). No equivalent standards are available for electrical mobility measurements. In conclusion, droplet size measurement for e-cigarettes can be readily achieved using commercially available sampling and measurement instrumentation, with suitable precision for product comparisons.
The impact of water pipe smoking on transitory changes in arterial stiffness

**Background:** Smoking is proven to be a major risk factor of the cardiovascular disease development and leads to the acceleration of arteriosclerosis within the coronary and peripheral arteries. Mechanisms playing an important role in the formation of these conditions among others include deterioration of arterial flexibility increasing their rigidity. The elastic properties of the arterial wall determine vascular adjustment responsible for accommodation to changes in the pulsatile blood flow. It is controversial whether the effects observed in smokers correspond to the nicotine or other substances in the smoke.

**Aim:** To compare cigarette and water pipe smoking and their effects on arterial stiffness and on changes in other hemodynamic parameters (pulse, systolic and diastolic pressure) and to evaluate the possible effect of CO on these changes (healthy subjects chosen).

**Material and Methods:** The study involved 30 water pipe smokers and 30 cigarette smokers and - to assess only the effects of nicotine - also 30 users of electronic cigarette (e-cigarette). Parameters changes were determined after 10 minutes of exposure. Arterial stiffness was measured by setting the stiffness index (SI) and reflection index (RI) using PulseTracePCA2 device (CardinalHealth, UK). Measuring the concentration of CO in exhaled air (COex) was performed using MicroCO device (MEDICOM, PL).

**Results:** Under the research conditions, there was no change of SI and RI among the water pipe smokers. Statistically significant changes were observed among cigarette and e-cigarette smokers. These changes apply only to RI, which increased in the case of the cigarettes smokers but decreased in case of the e-cigarettes users. COex concentration among the water pipe smokers increased 6-fold but among the cigarette smokers 2.5-fold.

**Conclusion:** Significant changes in hemodynamic parameters were observed only in relation to the cigarette smokers. There were no changes in arterial stiffness due to the water pipe smoking, while among its users the exposure to CO was significant.
Changes in hemodynamics and carboxyhemoglobin blood levels after use of electronic nicotine delivery systems among regular cigarette smokers

Smoking causes annually about 6 million deaths worldwide.

According to the report, the World Health Organization (WHO) to maintain unfavorable, the growing trend of tobacco use will cause, that since 2030 following the smoking habit approximately 8 million people suffer premature death each year.

A relatively new device, described by the producers as device to help smokers quit, is an electronic nicotine inhaler (e-cigarette). Its mission is to provide the body with small doses of nicotine, maintaining behavior of "ceremonial" smoking. Electronic cigarettes are the product of virtually unexplored in terms of actual effectiveness, and above all, their safety.

The aim of the study was to compare the effects of nicotine absorbed from conventional cigarettes and electronic cigarettes on changes in selected hemodynamic parameters. Due to the possibility of interaction of carbon monoxide contained in conventional cigarette smoke, and nicotine to changes in the studied parameters also intended to examine the changes in the concentration of carboxyhemoglobin after smoking cigarettes and using e-cigarettes.

Examined group consisted of 42 people, including 21 women and 21 men aged from 18 to 62 years, who declared daily cigarette smoking. Statistical analysis of the results was carried out by performing an analysis of variance (ANOVA).

In this study it was found, that as a result of conventional cigarette smoking increases all analyzed hemodynamic parameters, and statistically significant increases were observed for average diastolic blood pressure and heart rate (p <0.05). These increases are typically associated with nicotine absorbed by the smoker with smoke. It has also been a marked increase in carboxyhemoglobin, which is associated with a high concentration of carbon monoxide in tobacco smoke.

For use by the respondents of e-cigarettes has been observed slight increases in diastolic blood pressure and heart rate, but none of the parameters did not change significantly, indicating that either the use of e-cigarettes by the respondents did not provide the body with absorbable nicotine, or for the growth of haemodynamic parameters do not correspond to only nicotine, but also include other components that interact with the smoke nicotine to the smoker's organism.
Use of chiroptical spectroscopy to determine the ionisation status of (S)-nicotine in electronic cigarette formulations

In the vicinity of neutral pH, nicotine is equilibrated between unprotonated and monoprotonated states. The equilibrium is best described by the acid dissociation constant (Ka) and its logarithmic function known as pKa which in non-aqueous solution is termed psKa. E-cigarette formulations are predominately non-aqueous, typically composed of glycerol, 1,2-propanediol, water and (S)-nicotine, and the psKa of nicotine is influenced by the formulations’ concentration, solvent composition and temperature. The objective of this study was to determine the psKa of nicotine in a typical e-cigarette formulation and from that value calculate the distribution between the two states of nicotine. Circular dichroism spectra of (S)-nicotine change with pH, reflecting transitions between unprotonated and monoprotonated forms. Through addition of acids and alkali solutions we constructed titration curves at 20°C over the pH range 4 and 10, whose inflection points yielded the psKa value for nicotine under the specific ionic strength, co-solvent and temperature conditions of the (S)-nicotine/glycerol/water system. The concentration of (S)-nicotine was increased stepwise from 30μg/mL to 6mg/mL in each titration curve together with a concomitant reduction in cell path length from 10 to 0.01mm. From the six psKa values so obtained the psKa value was inferred at 30mg/mL (a typical e-cigarette formulation level). The psKa at 30mg/mL in 9% water balanced with glycerol at 20°C was estimated as 7.24. From this value, using the Henderson-Hasselbalch equation, the proportions of unprotonated and monoprotonated nicotine may be calculated with reference to the psH (the pH in co-solvent) of the formulation. The methodology described here is advanced as a robust approach for estimating the proportions of the two forms of nicotine that predominate in electronic cigarette formulations.
Bystander exposure to exhaled eVapour: assessment of nicotine in the ambient air using modelling and experimental approaches

There is currently a debate on whether vapour exhaled following the use of eVapour products has implications for the quality of air breathed by bystanders. A review of the current scientific literature indicates that there is insufficient evidence from which to assess the impact exhaled vapour has on indoor air quality.

A number of studies have reported that nicotine is exhaled into the air during use of eVapour products. We developed a model applying simple physical principles to assess the concentration of nicotine in ambient air. Herein, we consider an office environment, with a non-vaping worker seated 2m from a colleague who “puffs” once every 5 min over an 8 hour period (which includes a 1 hour lunch break). In this scenario, we assume the vaping worker inhales 0.06 mg of nicotine per puff, with 50% nicotine retention; both workers breathe at a rate of 8 L/min. The office is 37m3 with a typical air exchange rate of 50m3/h; this generates a movement by convection, so that each exhaled puff fills the room in 5 min. The cumulative effect under this scenario is reported.

From this model, we calculated that the total amount of nicotine inhaled by the non-vaping worker over the working day to be 22 µg, equivalent to 0.012 conventional cigarettes. In other words, the non-vaping worker would need to spend more than 3 months in this environment to inhale the equivalent amount of nicotine to smoking a single cigarette.

To refine and validate this model we present an experimental design for the assessment of indoor air quality before, during and after vaping under “real-life” conditions, covering other potential chemicals of interest. Taken together, appropriately validated models and robust experimental studies will assist in the development of evidence based regulation.
Risk assessing thermal breakdown and reaction products for E-cigarette flavours

The popularity of electronic cigarettes (e-cigarettes) is increasing. Flavours are an important aspect of the e-cigarette sensory experience. However, the unknown behaviour of flavour compounds when used in this application, including the potential thermal degradation due to the formulation contact with the hot coil, highlight the need for robust stewardship. Our toxicological risk assessment approach to flavours considers both the flavour itself and the identification, measurement and risk assessment of any potential thermal breakdown and/or reaction products. The initial flavour formulation is analysed and risk assessed, but here we focus on the risk assessment of potential flavour breakdown and reaction products.

The most important step of evaluation is to determine what is actually present in the aerosol that is delivered to the consumer. Accurate measurement using targeted analysis is required for specific compounds such as aldehydes and metals, resulting from thermal breakdown of humectants and contact with device materials respectively. However, because of the wide range of flavouring compounds and their potential reaction and breakdown products, the chemical profile of the delivered aerosol is better investigated using non-targeted, broad screening methods. We report semi-quantitative data obtained for e-cigarette aerosols using liquid injection Gas Chromatography (GC) linked to a Mass Spectrometer (MS) operated in scanning mode. Two different MS libraries (NIST and WILEY MS) were used to increase confidence in the peak assignations. Additionally Solid Phase Micro Extraction (SPME) was used to improve the sensitivity of compound identification.

Reaction products detected in the examples included propylene glycol acetal and ketal. Some thermal breakdown products from the humectants were observed as well as very low levels of substances that appeared to have leached from device material. Compounds were semi-quantified by the inclusion of mass-labelled surrogate standards and comparison of peak areas. Any compounds different to the formulation ingredients were risk assessed based on this semi-quantification.

If uncertainty in the risk assessment due to the semi-quantitative nature of the data precludes a conclusion on the supportability of the flavour, further work may be performed to measure any of the additional compounds identified in the aerosol more quantitatively.
Influence of machine-based puffing parameters on aerosol yields from e-cigarettes

Electronic Cigarettes are a new type of product rapidly gaining popularity with adult cigarette smokers. They typically produce an aerosol by evaporating a formulation containing nicotine and water with glycerol (VG), propylene glycol (PG) or a mixture of each. There is considerable interest in quantifying the emission levels of major and minor aerosol constituents from e-cigarettes. Despite this interest there are no formal agreed standards for the laboratory testing of e-cigarettes, such as selection of puffing machines, puffing parameters (e.g. puff volume or duration) or laboratory conditions, and a number of puffing regimes have been used to date.

The current study investigated the impact of machine parameters such as puff volume, puff duration and puff profile on the emissions of nicotine, glycerol and water from a disposable e-cigarette.

No statistically significant difference in emission levels of glycerol, water or nicotine were found between a rectangular puff profile and a bell-shaped profile, indicating that this experimental parameter did not play a significant role in driving levels of emissions. Similarly, puff volume did not influence emission levels. In contrast, puff duration was found to make a significant contribution to the magnitude of emissions, consistent with the importance of e-cigarette activation time in influencing emission levels. In contrast, emissions from combustible cigarettes show significant sensitivity to both puff volume and duration, consistent with the importance of oxygen supply in a combustion-based system.

The work in this study demonstrates significant differences in the importance of puffing parameters on the emissions from conventional cigarettes and e-cigarettes. As greater understanding of real-world e-cigarette puffing topography emerges, this study can support the development of standardised e-cigarette testing regimes.
Effect of nicotine on antioxidant status in HEMn-LP melanocytes

**Background**: Nicotine is a commonly used agent for smoking cessation therapies and promising substance in pharmacological attempts because of its presumed neuroprotective and antioxidant properties. It has been suggested that nicotine may be accumulated in human tissues containing melanin. Studies on mice revealed that pigmented tissues can store nicotine up to 30 days. This may in turn influence biochemical processes in human cells producing melanin. Melanin biopolymer is produced, stored and transported in melanosomes which are the specialized membrane-bound organelles of melanocytes. The role of melanin is to protect cells from UV radiation by creating a supranuclear cap in a cell, absorbing energy and working as an antioxidant agent and free radicals scavenger. Melanin is capable of binding many chemical substances, including drugs and nicotine.

**Aim**: The aim of this study was to examine the effect of nicotine on antioxidant status in cultured normal human melanocytes light pigmented (HEMn-LP).

**Methods**: The normal human epidermal melanocytes (HEMn-LP, Cascade Biologics) were exposed to nicotine in concentrations of 0.01, 0.05, 0.1, 0.5 or 1.0 mM for 24 h. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities, as far as hydrogen peroxide (H2O2) content were measured spectrophotometrically by the use of assay kits (Cayman, MI, USA and Cell Biolabs, Inc., USA).

**Results**: It has been demonstrated that the activity of SOD increases with rising concentration of nicotine. The treatment of cells with 0.1, 0.5 or 1.0 mM of nicotine, significantly increased the SOD activity by 10.7%, 13.1%, or 18.3%, respectively, as compared with the controls. The intracellular CAT activity was also increased by 10.3%, 18.0% or 25.2% for cells treated with nicotine in concentration of 0.1 mM, 0.5 mM or 1.0 mM, respectively. Treatment of melanocytes with 0.1, 0.5 and 1.0 mM of nicotine enhanced the H2O2 content by 13.8%, 25.5% and 37.0%, respectively, as compared with the controls. Nicotine in the concentration of 0.01 and 0.05 mM had no effect on cellular SOD and CAT activities as well as on H2O2 content. In contrast to SOD and CAT, nicotine had no statistically significant influence on the activity of GPx.

**Conclusion**: The obtained results may explain a potential ability of nicotine to induce oxidative stress in melanocytes in vivo, especially during long term exposition to nicotine, like cigarette smoking or use of nicotine replacement therapy (NRT).
Farsalinos KE, Kistler KA, Gillman G, Stefopoulos C, Spyrou A, Voudris V

Department of Cardiology, Onassis Cardiac Surgery Center, Athens, Greece.
Department of Chemistry, The Pennsylvania State University, Media, Pennsylvania, USA.
Enthalpy Analytical Inc., Durham, North Carolina, USA.

Evaluation of electronic cigarette liquids and vapour for the presence of selected inhalation toxins.

Introduction. Electronic cigarette (EC) liquids are available in a variety of food-grade flavorings. Some of them are known to be hazardous when inhaled. Two such examples are diacetyl (DA) and acetyl propionyl (AP), which are used for their buttery taste. Inhalation exposure causes a decline in respiratory function and the development of a rare clinical syndrome called obliterative bronchiolitis. The purpose of this study was to evaluate sweet-flavoured EC liquids for the presence of DA and AP.

Methods. In total, 159 samples (refill liquids and concentrated flavors) were purchased from 36 manufacturers and retailers from 6 European countries and the US, and were tested for the presence of DA and AP. Additionally, three liquids were prepared by dissolving a concentrated flavor sample of known DA and AP levels at 5%, 10% and 20% concentration in a mixture of propylene glycol and glycerol. The vapor produced from these liquids using an EC device was analyzed to determine the concentration of DA and AP.

Results. DA and AP were found in 74.2% of the samples, with more samples containing DA. The median daily exposure levels were 56μg/mL (IQR: 26-278μg/mL) for DA and 91μg/mL (IQR: 20-432μg/mL) for AP. They were 2-times higher than the strict NIOSH-defined safety limits but 100 and 10 times lower compared to smoking respectively. Similar concentrations were found in liquid and vapor for both chemicals, with a very strong correlation between the concentration in liquid and in vapor.

Conclusions. DA and AP were found in a large proportion of sweet-flavoured EC liquids, at levels that were higher than the strictest safety limits but significantly lower compared to smoking. The presence of these chemicals in EC liquids represents an avoidable risk. Proper measures should be taken by EC liquid manufacturers and flavouring suppliers to eliminate these hazards from the products.
Nicotine absorption from electronic cigarette use: comparison between experienced consumers (vapers) and naïve users (smokers).

Background. Electronic cigarettes (ECs) are novel products in tobacco harm reduction. Their potential to deliver nicotine is not clear. The purpose of this study was to evaluate plasma nicotine pharmacokinetics and compare experienced EC consumers (vapers) with naïve users (smokers).

Methods. In total, 47 subjects participated to the study, 24 experienced vapers (all former smokers) and 23 smokers who had never used an EC before. A new generation EC device was used, with a clearomizer-type atomizer and an 18mg/ml nicotine-containing liquid; the power was set at 9 watts. After abstaining from tobacco and EC use, food, alcohol and coffee intake for at least 8 hours, subjects took 10 puffs with a 30 second interpuff interval for the first 5 min; then, they used the EC ad lib for an extra 60 min (total use time of 65 min). Blood samples were obtained at baseline, 5 min, 20 min, 35 min, 50 min and 65 min time points.

Results. Both groups has similar age (40 ± 10 years vs. 40 ± 9 years, P=NS) and smoking duration (21 ± 10 years vs. 21 ± 10 years, P=NS). At baseline, no difference in plasma nicotine levels was found. Subsequently, significant differences were observed at all time points, with experienced vapers showing significantly higher plasma nicotine levels compared to smokers. The highest difference was observed at 20 min (117% higher levels in vapers compared to smokers). At 65 min, plasma nicotine levels were 24.09 ng/ml (SEM: 1.96) in vapers and 13.83 ng/ml (SEM: 1.63) in smokers (P<0.001). The levels after 65 min of ad lib use in smokers were lower compared to smoking one tobacco cigarette in 5 minutes.

Conclusions. The results indicate that naïve users exhibit lower and slower nicotine absorption from EC use compared to experienced users. There is minimal possibility of nicotine overdose in naïve users, and ECs seem to have less addictive properties compared to smoking. However, nicotine delivery needs to be substantially improved so that they become more successful in substituting smoking.
Medical University of Silesia, Poland

**Nicotine concentration in liquids and cartridges for electronic cigarettes available on the polish markets**

**Significance**: Electronic nicotine delivery devices (ENDS), commonly called electronic cigarettes or e-cigarettes, have gained popularity as an alternatives for traditional cigarettes. They are portable devices used to inhale small doses of nicotine. Although e-cigarettes are widely sold on-line and by retailers. New research suggests that they may contain unexpected toxins and may provide unreliable nicotine delivery.

**Aim**: The aim of the study was to measure nicotine concentration in liquids and cartridges used in e-cigarettes.

**Materials and Methods**: Nineteen e-liquids and sixteen cartridges were chosen based on their popularity on the Internet. For nicotine analysis in e-liquids, was collected 1 ml of each solution, added 100μl of quinoline (internal standard), and assayed with gas chromatography method (GC-TSD). Nicotine from cartridges was extracted with 100ml of methanol with internal standard for 30 minutes in an ultrasonic bath. Precision of both methods was 10%.

**Results**: Nine liquids had significantly different nicotine level than declared by retail sellers. Differences of the same magnitude were also detected in nine cartridges. In extreme cases, detected nicotine levels in e-liquids and cartridges were lower than declared by 72% and 91%, respectively.

**Conclusions**: Nicotine content in liquids and cartridges used in electronic cigarettes significantly varied from values declared by their distributors. Quality control of electronic cigarettes (ENDS) should be implemented in the future.
Comparison of the general condition and respiratory system function in smokers and nonsmokers based on spirometry

**Significance**: Spirometry is a basic test of lung function. It is of particular importance in the detection of respiratory dysfunction in smokers. The main disease that can occur in these patients and which is detected by spirometry is chronic obstructive pulmonary disease. The decrease in spirometric parameters suggests that negative changes in the lungs and airways. Although the number of smokers, slightly but steadily decreasing, then there are other alternative forms of nicotine delivery the body such as water pipe, snuff and electronic cigarettes. Safety assessment of their use must include their effect on lung function and compare the effects of tobacco smoke should give an answer to the question of whether those products safer than conventional cigarettes.

**Aim**: The aim of this study was to determine whether differential parameters obtained by spirometry before and after smoking a conventional cigarette is so important to apply this method to the evaluation of lung function after using the electronic cigarette.

**Materials and Methods**: In the study participated 82 people, men and women, smokers and non-smokers. In the group of area due to the criterion of being before the first morning cigarette excluded 32 people. Nonsmoker group performed spirometry and burning two studies: the concentration of exhaled carbon monoxide and spirometry before and after smoking a cigarette. Also collected from smokers detailed information on their addiction through appropriate surveys.

**Results**: Among the parameters only FVC parameter changes in the control group, before and after a cigarette, in females and males were statistically significant. It was also found that the values of two parameters, namely, FVC, and FEV1/FVC are strongly correlated with the concentration of carbon monoxide formed during the combustion of the cigarette.

**Conclusions**: Smoking cigarettes strongly influence on peak expiratory flow (PEF). FEV1/FVC are strongly correlated with the concentration of carbon monoxide. There are statistically significant correlations.
Feellife’s Research Survey on Nicotine

Nicotine is one kind of the more than 40 alkaloids contained in tobacco. Among these alkaloids, nicotine is with the most content, and it is the most widely used and the most deeply researched. Nicotine has protective effects on the incidence of Parkinson’s disease. At the same time, nicotine is widely used in electronic cigarettes, pesticide, and medicine. Feellife company has been focusing on the following four aspects over the past few years.

1. Study on nicotine production technology. Feellife has developed a MS-RTF technological process with higher recovery rate and higher purity. As a result, the purity and recovery of our production have improved a lot.

2. Study on nicotine determination method. Feellife has developed a method for the determination of nicotine content in a fast, accurate, and stable way.

3. Study on the medicinal effects of nicotine. Since 2012, Feellife started a cooperation with Shenzhen Research Institute of Tsinghua University, on studying the effects of nicotine on Parkinson's disease and Alzheimer's, and speculated that the mechanism should work with nicotine activating nicotinic acetylcholine receptor, so as to develop neural inflammatory responses.

4. Study on safety of nicotine use and application. Feellife started to study the security assessment of ingredients of e-liquid vapor from the beginning of 2013. Feellife improved the existing e-liquid products and enhanced the safety according to the safety information data. At the same time, Feellife developed nicotine DIY solution, liquid nicotine, nicotine inhalers and other products.
PRELIMINARY RESEARCH ON THE VIABILITY OF HUMAN LUNG ALVEOLAR EPITHELIAL CELLS A549 EXPOSED TO ELECTRONIC CIGARETTE VAPOR EXTRACT AND ELECTRONIC CIGARETTE LIQUIDS

Background: Electronic cigarettes (e-cigarettes) were designed to imitate conventional cigarettes. The idea of the e-cigarettes is to deliver nicotine into the smoker’s body, but without the toxic substances. The research conducted to date on the content of toxic substances shows that in the aerosol generated by e-cigarettes toxic components are present alongside nicotine(1, 2). In the aerosol carbonyl compounds, volatile organic compounds, nitrosamines and heavy metals were detected, however they were present in lower concentrations than in the smoke generated by conventional cigarettes. Currently, research is conducted on the influence of the aerosol generated by the e-cigarette on cell survival ratio.

Aim: The aim of the presented work was to examine the viability of human lung alveolar epithelial cells A549 exposed to electronic cigarette vapor extract and electronic cigarettes liquids.

Methods: The human lung alveolar epithelial cells (A549, Sigma Aldrich) were used. The viability of A549 cells was evaluated by WST-1 colorimetric assay. The aerosol was generated from e-cigarettes using the “Palaczbot” device (Lodz University of Technology). It rinses the complete culture medium as necessary to obtain the extract. Aerosol was generated in the following conditions: puff duration – 2 seconds, break between puffs – 60 seconds and puff volume – 50 ml. In the study filtered extracts were used with concentrations between 100 and 0,098%, obtained by dilution of the stock solution.

Results: The survival ratio of cells exposed to the extract obtained from convectional cigarettes in case of the concentration of 100% was 0,85 and up to 100 in case of the concentration of 0,195%. In contrast, the survival ratio with the use of extract obtained from the e-cigarettes ranged from 0,87 in case of 100% concentration, glycerol/propylene glycol basis and voltage of 4,08 up to 98,6 in case of the concentration of 0,098%, glycerol/propylene glycol liquid and voltage of 4,0 in comparison to the control group.

Conclusion: The results indicate that the survival ratio decreases with the increase of both the e-cigarette and the conventional cigarette aerosol concentrations.
METABOLISM OF NICOTINE AMONG CAUCASIAN SMOKERS

Significance: Sex is a factor which mediates the neurobiological response to nicotine. Also is a course of dependence behavior in humans that smoke cigarettes. Basic science research has demonstrated nicotine-induced sex differences in animal models by measuring various behavioral and non-behavioral indices. Epidemiological research also strongly suggests sex differences in tobacco use and abuse.

The aim of the study: Characterize rate of nicotine metabolism among Polish smokers.

Materials and Methods: 187 regular cigarette smokers (85 males), collected urine samples and measured 3’-trans-hydroxycotinine and cotinine levels with LC-MS/MS method. We calculated Nicotine Metabolite Ratio (NMR), a phenotypic and noninvasive indicator of nicotine metabolism rate, defined as a molar ratio of two major metabolites of nicotine: trans-3’-hydroxycotinine over cotinine. We analyzed variations in NMR among various groups of smokers using analysis of variances with sex and age as independent variables.

Results: The NMR was 4.77 (95% CI: 4.35; 5.22). Statistical analysis revealed significant difference between the group of women below 40 years, and the rest of the female population (6.20; 95%CI: 6.12; 7.91 vs. 4.47; 95% CI: 4.42; 5.91). No statistical changes were found in male smokers of various ages.

Conclusions: Young Caucasian women metabolize nicotine faster than the rest of population. The increased nicotine metabolism in female relative to male smokers may be mediated by circulating gonadal hormones. Further studies are needed to assess whether young Caucasian women require higher doses of NRT during smoking cessation treatment.
Quitting methods among former and current smokers - a study among callers to a smoking quit line and users of a smoking cessation web page

Background

Several smoking cessation aids are available for smokers wishing or trying to quit, and recent additions to support smokers are quit-apps and e-cigarettes. The aim of this project was to map out smoking cessation methods among quit-line callers and users of a smoking cessation web-page.

Methods

Study participants were 16 years or above, and were either current smokers in a quitting/reducing process, planning to quit/reduce, or former smokers who search help to avoid relapse. An electronic questionnaire with forced choice and skip patterns was used. The data presented here consist of information from 629 men and women from the baseline study (Nov 2013- May 2014). 115 respondents were recruited via the telephone quit line while 514 were recruited via the web page. 48 non-smokers were excluded from the analyses, resulting in an analytical sample of 581 subjects. The study is planned with two follow-ups.

Results

403 women and 226 men filled in the baseline questionnaire. Out of the 203 current smokers, 179 were currently trying to quit or reduce smoking. Among current quitters/reducers, 29% used different types of web/app/social media (4 items), 27% used e-cigarettes and 27% used NRTs (4 items). Prescription medication was reported used by 12%, while 10% used snus. Cessation methods used in earlier quit attempts among current smokers were also registered, with NRTs as the most used method (61%), followed by Apps/Web-pages (27%) and prescription medication (25%). 23% reported use of e-cigarettes and 21% use of snus. Among the 378 former smokers, the most common method used was NRTs (61%), e-cigarettes (58%) and applications/web pages (50%). 29% reported the use of prescription medication, while 30% reported the use of snus as a method to quit.

Conclusion

The most used quitting method among current quitters/reducers in this study was the use of applications/webpages, NRTs and e-cigarettes. The most reported method used by unsuccessful quitters in their previous attempts was NRTs. Former smokers reported mainly use of app/web, e-cigarettes and NRTs (50% or more). The results from the present study indicate that the use of traditional quitting aids like NRTs has been ousted by e-cigarettes in Norway, and also that e-cigarettes are competing with the overall use of NRT products.
Non-Combustible Alternatives: Assessing Potentially Reduced-Risk Products – Toxicological and Clinical Exposure Studies

OBJECTIVE: Providing adult smokers, who would otherwise continue smoking, with reduced risk alternatives to combustible cigarettes is the basis of tobacco harm reduction. Philip Morris International (PMI) is developing a portfolio of potentially reduced risk products (pRRPs) that reduce the formation of harmful and potentially harmful compounds (HPHCs) found in cigarette smoke, primarily by eliminating combustion of tobacco. Our dual objectives are to develop products that (1) significantly reduce the risk of smoking-related diseases and (2) are accepted by smokers as cigarette substitutes. Data evaluating the reduced risk potential of a “heat-not-burn” prototype pRRP have been generated.

METHODS: Analytical chemistry data were generated using ISO 17025 accredited methods. In vitro toxicological assays were conducted according to GLP. Exploratory clinical studies to measure biomarkers of HPHC exposure (BoExp) in humans were conducted using a 5 day open-label, randomized, controlled, 2-arm parallel group design, in confinement in 40 healthy smokers aged between 21 and 65 years. Smokers used the pRRP without restriction (ad libitum), and dual use of combustible cigarettes (CC) and the pRRP was not permitted. After study admission, subjects smoked CC for 2 days (baseline) and were subsequently randomized to continuing to smoke CC, or switched to the prototype pRRP for 5 consecutive days. BoExp were measured using validated methods. This study was conducted according to GCP and is registered in ClinicalTrials.gov, number NCT01780714.

RESULTS: Analytical chemistry assessment showed substantial reductions in HPHCs on a per-unit and a per-unit-nicotine basis when compared to CC controls. In vitro toxicological assays comparing the toxicity of the pRRP aerosol to CC smoke showed substantial reductions in biological activity. At the end of the clinical 5-day exposure study, the reduction in levels of primary BoExp in the pRRP arm as compared to CC, ranged from 72.1% to 93.0%. Differences between the 2 arms were statistically significant and were observed within 24 hours of starting use of the pRRP.

CONCLUSIONS: Compared to CC, the aerosol chemistry of the prototype pRRP showed substantial reductions in the formation of HPHCs, resulting in reductions in biological activity measured using standard toxicological assays. In a clinical setting, exposure to HPHCs was significantly reduced in subjects switching from CC to the prototype pRRP, as compared to subjects who continued smoking CC for 5 days. Although these data indicate potential, a range of further studies, including under conditions of actual use, are planned to assess reduced risk.
Assessing the Pharmacokinetic and Pharmacodynamic Profiles of Non-Combustible Potentially Reduced Risk Products

OBJECTIVE: Providing adult smokers who would otherwise continue smoking with reduced risk alternatives to combustible cigarettes is the basis of tobacco harm reduction. Philip Morris International (PMI) is developing a portfolio of potentially reduced risk products (pRRPs) that reduce the formation of harmful and potentially harmful compounds (HPHCs) found in cigarette smoke, primarily by eliminating combustion of tobacco. Our dual objectives are to develop products that (1) significantly reduce the risk of smoking-related diseases and (2) are accepted by smokers as cigarette substitutes. Data has been generated in an exploratory clinical study to evaluate the pharmacokinetic (PK) profile of nicotine following single use of a “heat-not-burn” pRRP prototype as compared to conventional cigarettes (CC). This study is part of an on-going program to determine the extent to which adult smokers would find the pRRP an acceptable substitute for combustible cigarettes, recognizing that other factors such as taste and product design are important in determining consumer acceptability.

METHODS: Nicotine PK and urge to smoke were measured in an open-label, randomized, two-period, two-sequence crossover study in 28 healthy smokers. Each period consisted of 3 days, with 1 day of smoking abstinence, 1 day with a single use, and 1 day with ad libitum use of a prototype pRRP or CC. During the single use day, a total of 16 venous blood samples were collected. Urge-to-smoke was assessed using the Questionnaire of Smoking Urges Brief (QSU-b). Safety was monitored throughout the study. This study was registered with ClinicalTrials.gov, number NCT01780688.

RESULTS: The prototype pRRP nicotine PK profile in plasma was similar to combustible cigarettes. The time to maximal nicotine absorption for both products in the study was 8 minutes, the maximum nicotine concentration was 70% that of a combustible cigarette, and the total nicotine absorption was at 77%. The study also found that the urge to smoke profile was similar to combustible cigarettes.

CONCLUSIONS: The PK and PD profiles were similar for both CC and the prototype pRRP due to absorption at the pulmonary level. Although these data indicate potential, a range of further studies, including under conditions of actual use, are planned to assess the acceptability of the pRRP.
Tobacco Regulation, smoking prevalence and the implications for E-Vapour Products

The primary focus of tobacco regulatory policy is to reduce smoking rates. The European Tobacco Product Directive was introduced this year with the goal of reducing prevalence across Europe by 2% over five years; Australia has made reduction of its smoking rate to 10% by 2018 a key target within its National Strategy; and Ireland and New Zealand have each pledged to reduce smoking rates to under 5% by 2025.

This paper will evaluate the extent to which these goals are achievable, and will investigate the facts behind the hype to consider what impact initiatives such as smoking bans, taxation, and the recent introduction of plain packaging in Australia have really had on smoking rates.

The decline in smoking rates has been levelling off for the last ten years in most developed countries, including Ireland, Australia, Canada and the UK which are all held to be leaders of tobacco regulation. According to data reported in 2013 by the Organisation for Economic Co-operation and Development (OECD), Greece and Ireland have the highest smoking rates in the European Union, whereas Sweden has the lowest – at just 12.5% for men and 14.3% for women in 2011. A new OECD survey is expected later in 2014 and it will be interesting to see whether these trends have continued.

As far as adult smoking rates are concerned, there has been a consistent decline in smoking in countries such as Sweden and Norway where snus is widely available. Not only are the current smoking rates already low, but these rates of decline in smoking are continuing. The Swedish and Norwegian experiences may be the best indicators of the potential for the E-Vapour sector in the US and Europe. However, maintaining the current rate of growth depends greatly on the pace of regulation. It is most important that regulation continues to differentiate E-Vapour products from tobacco products, especially in terms of their continued availability and visibility to adult consumers.
ACUTE EFFECTS OF ELECTRONIC NICOTINE DELIVERY SYSTEMS (ENDS) ON ARTERIAL STIFFNESS IN HEALTHY CIGARETTE SMOKERS

**Significance**: Electronic nicotine delivery systems (ENDS), commonly called electronic cigarettes or e-cigarettes, are cigarette-like plastic devices that generate vapor when inhaled by a user, by means of heating a nicotine solution in a mixture of propylene glycol and water. Little is known about their safety, quality, and potential health effects.

**Aim**: The aim of the study was to investigate the acute effect of electronic (ENDS) and conventional cigarettes on arterial stiffness.

**Materials and Methods**: The study was performed using a randomized placebo-controlled double blind cross-over design. Sixteen healthy smokers, 6 men and 10 women, aged 37±13 years, were recruited and studied in the morning on two different days. All subjects were regular smokers who smoked >10 cigarettes per day. In each session, participants were randomly provided with an ENDS with a 16 mg nicotine cartridge or ENDS containing a cartridge without nicotine (placebo). Subjects were also asked to smoke their regular cigarette one hour after the use of ENDS. Stiffness Index (SI) and Refection Index (RI) were measured using Pulse Track monitor at the beginning of the experiment, 15 and 60 minutes after the use of ENDS, and 15 and 60 minutes after smoking a conventional cigarette. The effect of nicotine or placebo from e-cigarette on arterial stiffness was described as the relative change from baseline values. A two-way ANOVA for repeated measures was used to detect changes in arterial stiffness over time.

**Results**: Single use of ENDS was not associated with changes in arterial stiffness. We did not observe any significant differences in SI and RI after single use of ENDS with nicotine when compared with placebo (4.0±4.8 vs. –3.2±5.0% and 8.9±6.0 vs. 11.0±9.9%, respectively; p >0.05). No significant differences in SI and RI were detected after single use of ENDS with nicotine when compared to conventional cigarettes (4.0±4.8 vs. 7.0±7.3% and 8.9±6.0 vs. 3.2±5.8%, respectively; p >0.05).

**Conclusions**: We did not observe acute effects of ENDS on arterial stiffness in healthy cigarette smokers.