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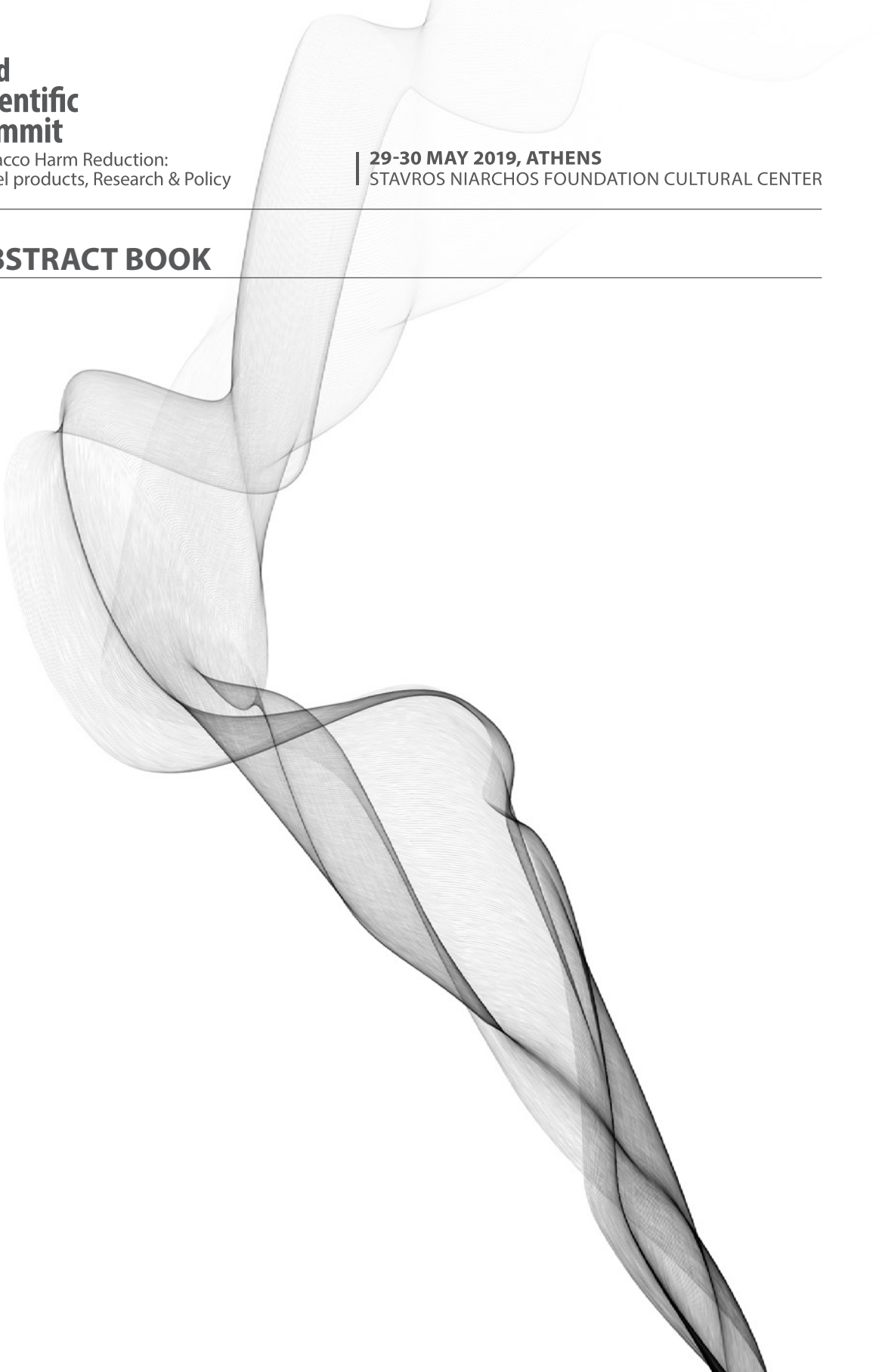
ABSTRACT BOOK



ABSTRACT BOOK

EPIDEMIOLOGY & SOCIAL ISSUES (PERCEPTION RISK, ATTRACTIVENESS ETC.)

ELECTRONIC CIGARETTE USE AMONG ADOLESCENTS IN GREECE: A CROSS-SECTIONAL SCHOOL STUDY IN THE METROPOLITAN AREA OF ATHENS

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Background: Electronic cigarette use among adolescents has been steadily increasing over the last decade. At the same time, the use of electronic cigarettes is still not regulated in many countries, nor are there restrictions regarding the sale of electronic cigarettes to children and adolescents. Multiple questions arise regarding the overall burden associated with the use of electronic cigarettes.

Aim of this study is to investigate the prevalence of electronic cigarette use among adolescent pupils in our country and its correlations with a range of risk and/or protective factors.

Methods: A cross-sectional study in public and private, both general and vocational, high schools in the metropolitan area of Athens. Students from all three high school classes were invited to participate. Participation was voluntary and anonymous, while consent was actively obtained from both pupils and their parents. A self-completed questionnaire was administered to pupils in the classroom, with a completion time of approximately 20 minutes. We investigated the prevalence, as well as some descriptive characteristics (age of onset, frequency etc.) of the use of electronic and conventional cigarettes. Possible risk and/or protective factors investigated in our study included socio-demographic and socio-economic factors, mental and physical health indicators, as well as variables regarding exercise and social media use.

Results: We are going to present some preliminary findings from the first phase of our study. During this phase we recruited a sample of 800 pupils from 4 public and 1 private high school.

Conclusions: Epidemiological studies at a national level that reflect the current situation regarding the growing use of this new form of cigarette are needed so that appropriate intervention and prevention strategies could be designed and implemented.

ABSTRACT BOOK

EPIDEMIOLOGY & SOCIAL ISSUES (PERCEPTION RISK, ATTRACTIVENESS ETC.)

PATTERNS OF USE, PAST SMOKING STATUS AND BIOCHEMICALLY VERIFIED CURRENT SMOKING STATUS OF HEATED TOBACCO PRODUCT (IQOS) SHOPS CUSTOMERS: PRELIMINARY RESULTS

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Background: The purpose of this study is to examine the characteristics and past and current smoking status in customers of shops selling heated tobacco products (HTPs, IQOS) in Athens, Greece.

Methods: Researchers visited 2 IQOS stores. Participants were consecutive customers who were buying products for own use. They answered a questionnaire, while current smoking status was verified by measuring exhaled carbon monoxide (eCO). Current smokers were those who reported currently smoking tobacco cigarettes and had eCO≥7 ppm. Former smokers were those who reported past smoking history and had eCO levels<7 ppm.

Results: Participants (N=174) were mostly current and former smokers (97.1%). Five participants reported never smoking; all had eCO<7ppm. More participants were former (60.3%) rather than current smokers (36.8%, P<0.001); no difference was found in smoking consumption before initiating HTP use (21±11 cigarettes for current and 21±15 for former smokers). Former smokers were using the HTP for longer (12.1 months) compared to current smokers (8.3 months, P=0.016). More former (93.3%) compared to current smokers (77.4%) were using HTP daily, with never smokers reporting lower daily consumption compared to the other groups (18±11 sticks for current, 19±11 for former and 8±6 for never smokers, P=0.038).

Conclusions: HTP (IQOS) store customers are predominantly current and former smokers, with the majority having quit smoking. A small minority are never smokers who do not subsequently initiate smoking. Clinical implications: The findings indicate that the majority of HTP users are smokers using them as substitutes for smoking. Long term epidemiological studies are needed to determine the health effects of using HTP products.

ABSTRACT BOOK

EPIDEMIOLOGY & SOCIAL ISSUES (PERCEPTION RISK, ATTRACTIVENESS ETC.)

RISK PERCEPTION AND APPEAL OF DIFFERENT TOBACCO HARM REDUCTION PRODUCTS (THRs) AMONG 100 PARTICIPANTS, BETWEEN 18-24 YEARS OLD IN WESTERN GREECE

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Background: Cigarette smoking is identified as an important source of preventable morbidity and premature mortality according to World Health Organization (WHO). It has always been addictive for every age and social group. Greece is the country with the highest smoking prevalence in Europe.

Harm reduction products such as electronic cigarettes (ECs) and heat not burn (HnB) products (e.g. Glo, IQOS), show increase in popularity and promise to provide smokers reduced-risk alternatives to cigarettes and other forms of combustible tobacco. The public health effects of harm reduction products such as IQOS depend on the balance between being appealing to smokers and minimally attracting non-smokers.

Methods: The aim of this survey was to examine the perceptions of health risks of harm reduction products such as IQOS, the perceived harm to others and the addiction that users have. To address this research questions, a survey was performed via questionnaires, in order to assess the risk perception and the appeal of tobacco cigarettes, electronic cigarettes and IQOS among 100 participants between 18-24 years old in Western Greece. Different subsections addressed risk perception and appeal of each product, using similar questions. A modified version of the Perceived Risk Instrument (PRI; PMI, 2016) has been used to compare respondents' perceptions of the health and addiction risks associated with each of three tobacco/nicotine use behaviors: (i) smoking cigarettes; (ii) using e-cigarettes; IQOS; nicotine replacement therapies instead of smoking cigarettes; (iii) quitting all tobacco and nicotine use completely; never starting to smoke cigarettes. Each section of the questionnaire begins with a brief description of the tobacco/nicotine product/behavior and how it works. Respondents repeated this process for each of the other three tobacco/nicotine use behaviors. The Results are presented after a descriptive analysis, with continuous variables expressed as median (IQR) and categorical variables as number (%), with 95% CIs.

ABSTRACT BOOK

ALDEHYDE CONTENT IN THE VAPOUR PRODUCED BY GLO

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Heat not Burn (HnB) devices have emerged in the recent years as a safer alternative to conventional cigarettes. Instead of combusting the tobacco, they produce a vapor, in lower temperatures, producing fewer toxicants than cigarette smoke. Tobacco sticks are inserted to the holder containing a heating element that causes steam to be produced by the tobacco in lower than combustion temperatures. One device using this technology is Glo.

One of the main health concerns about HnB usage is the potential for toxic carbonyl emissions since these compounds are potential irritants, toxicants and/or carcinogens.

In this study, we measured the emission level of aldehydes (formaldehyde, acetaldehyde, crotonaldehyde and acrolein) present in Glo vapor. The results were then compared with their levels produced by combustible cigarettes, since these compounds are quite prevalent in conventional tobacco smoke.

Our study has shown much lower aldehyde emissions in the Glo vapor.

ABSTRACT BOOK

THE EFFECT OF VAPOR PRODUCED BY E-CIGARETTE AND HnB DEVICES ON EFFECTIVENESS OF KANAMYCIN

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Background: A plethora of electronic cigarette and Heat not Burn (HnB) devices are widely available to the consumers as an alternative to the use of conventional cigarettes[1,2]. The use of said devices has been shown to possibly lead to physiological changes to the user, such as oxidative stress[3]. In addition it can lead to higher risk of lung inflammation and pulmonary infections, either bacterial or viral[4].

One of the most important factors into successfully managing a pulmonary infection is the administration of antibiotics.

Aim – Methods: The aim of this study was to explore the effect the presence of vapor produced by e-cigarettes, as well as HnB devices (IQOS, Glo), on the effectiveness of kanamycin, when used to inhibit the bacterial growth in e-coli cultures. In addition the results were compared to the corresponding results produced using conventional cigarette smoke.

References

1. *J.K. Pepper, N.T. Brewer, 2013, Electronic nicotine delivery system (electronic cigarette) awareness, use, reactions and beliefs: a systematic review, Tobac. Contr., 23, pp. 375-384.*

2. *Smith et al., 2016, Evaluation of the Tobacco Heating System 2.2. Part 1: description of the system and the scientific assessment program, RTP (Regul. Toxicol. Pharmacol.), 81 (Suppl. 2), pp. 17-26.*

3. *G.I. Andrikopoulos, Z. Zagoriti, S. Topouzis, K. Poulas1, Oxidative stress induced by electronic nicotine delivery systems (ENDs): Focus on respiratory system, Current Opinion in Toxicology (2018), doi: 10.1016/j.cotox.2018.09.002.*

4. *Korfei, M. (2018). The underestimated danger of E-cigarettes - also in the absence of nicotine. Respiratory Research, 19(1). doi:10.1186/s12931-018-0870-4.*

ABSTRACT BOOK

BIOMARKERS' EVALUATION IN ANIMAL OR HUMAN STUDIES

ELECTRONIC CIGARETTE SMOKING INCREASES ARTERIAL STIFFNESS AND OXIDATIVE STRESS TO A LESSER EXTENT THAN A SINGLE NORMAL CIGARETTE: AN ACUTE STUDY

Ignatios Ikonomidis[2], **Dimitrios Vlastos**[2], Gavriella Kostelli[2], Kallirrhoe Kourea[2], Ourania Kondylopoulou[2], Stefanos Vlachos[1], Dimitrios Benas[1], Maria Varoudi[1], Georgios Pavlidis[1], Vasiliki Dede[1], Heleni Triantafyllidi[1], Ioanna Andreadou[1], John Lekakis[1]

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Background: Electronic cigarette is proposed as a bridge to smoking cessation. In this study we examined its acute effects on aortic elasticity, exhaled CO concentration, and oxidative stress in comparison with a conventional cigarette.

Methods: Seventy current smokers (mean age 48 years±5) without cardiovascular disease participated in the study. The subjects were randomized to smoke either a single normal or an electronic cigarette for 7 min (simulating the duration of a normal cigarette smoking), and after a 40 minute washout period were crossed over to the alternative cigarette. Of the 70 subjects, 35 used a nicotine-free electronic cigarette fluid, while the remaining 35 an electronic cigarette fluid with a nicotine concentration of 12 mg/dL. Measurements were performed at baseline and after smoking the normal or electronic cigarette, including: a) the aortic PWV (PWV) and augmentation index (AIx) by Arteriograph and Complior; b) the exhaled CO level (parts per million-ppm) as a smoking status marker; and c) the plasma malondialdehyde (MDA) levels as an oxidative stress burden index.

Results: Compared to baseline, PWV, exhaled CO, and MDA levels were lower after the electronic than after the conventional cigarette smoking (9.3±0.2 vs 10.3±0.2 vs 10.8±0.2 m/s, $p<0.05$; 12.9±0.7 vs. 12±0.6 vs 14.9±0.7 ppm, $p< 0.001$; 1.09±0.1 vs 1.17±0.1 vs 1.28±0.1 μmol/L, $p<0.001$, respectively). In addition, nicotine-free electronic cigarette caused a significantly smaller increase of arterial stiffness, compared to the nicotine containing fluid (ΔPWV=+0.3 vs +0.9m/s, $p<0.05$, respectively).

Conclusions: Electronic cigarette smoking causes a smaller increase of arterial stiffness and oxidative stress, compared to a single normal cigarette in an acute setting. This might imply that it could serve as a safer bridge to smoking cessation.

ABSTRACT BOOK

ELECTRONIC CIGARETTE SMOKING INCREASES ARTERIAL STIFFNESS AND OXIDATIVE STRESS TO A LESSER EXTENT THAN A SINGLE NORMAL CIGARETTE: A CHRONIC STUDY

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Background: Electronic cigarette is proposed as a bridge to smoking cessation. In this study we examined its effects on aortic elasticity, exhaled CO concentration, and oxidative stress after 1 month of use.

Methods: Seventy current smokers (mean age 48 years ±5) without cardiovascular disease participated in the study. All 70 subjects smoked an electronic cigarette with nicotine concentration of the fluid of 12 mg/dL for one month. Measurements were performed at baseline and after 1 month of electronic cigarette smoking. We measured a) the aortic PWV (PWV) and augmentation index (AIx) by Arteriograph and Complior; and b) the plasma malondialdehyde (MDA) levels as an oxidative stress burden index.

Results: We observed a significant improvement of AIx, as well as of MDA compared to baseline (25.6±2% vs 29.5±2%, p= 0.01; 1.09±0.1 vs 1.22±0.1 μmol/L, p<0.05 respectively).

Conclusions: Replacement of normal cigarettes by a moderate nicotine concentration electronic cigarette results in improved aortic elasticity and oxidative stress within 1 month.

ABSTRACT BOOK

ELECTRONIC CIGARETTE SMOKING CAUSES LESS IMPAIRMENT OF PLATELET FUNCTION AND OXIDATIVE STRESS THAN TOBACCO CIGARETTE SMOKING

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Background: In smokers, chronic platelet overactivation may cause reduced platelet adhesion and aggregation *in vitro* reflecting platelet dysfunction. Electronic cigarette is proposed as a bridge to smoking cessation. We examined its effects on platelet function after 1 month of use compared to tobacco smoking.

Methods: Forty current smokers (mean age 48 years±5) without cardiovascular disease were randomized to smoke either a convention tobacco (conv-cig) or an electronic cigarette (e-cig) for one month and then were crossed over to the alternative cigarette (e-cig or conv-cig). All subjects smoked an electronic cigarette with nicotine concentration of the fluid of 12 mg/dL for one month. Measurements were performed at baseline and after one month of smoking the conventional or electronic cigarette We measured a) the aortic PWV (PWV) and augmentation index (AIx) by Arteriograph and Complior ; b) the exhaled CO level (parts per million-ppm) as a smoking status marker c) the plasma malondialdehyde (MDA) levels as an oxidative stress burden index; d) platelet function by two different methods, namely the novel Platelet Function Analyzer PFA-100 and the traditional Light Transmission Aggregometry (LTA). The PFA-100 evaluates high-shear stress dependent platelet function based on a cartridge system in which the process of platelet adhesion and aggregation following a vascular injury is simulated *in vitro*.

Results: After 1 month of electronic smoking, we observed a modest but significant improvement of AIx and platelet function as assessed by PFA (normal value >142 U) and LTA (normal response for ADP >63%, epinephrine (EPI) >54% and collagen (COLL) >61%), as well as of MDA and CO (14.9±0.78 vs 19±0.6 vs 8.6 ±0.7 ppm), compared to baseline and conventional tobacco smoking ($p<0.05$ table).

Conclusions: Electronic cigarette smoking causes a modest improvement of wave reflections, platelet function and oxidative stress compared to tobacco cigarette smoking within 1 month of use.

ABSTRACT BOOK

EVALUATION OF ANXIETY BEHAVIOR AND CHOLINESTERASE'S ISOFORMS ACTIVITY OF ADULT MICE AFTER CIGARETTE SMOKE EXPOSURE

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Background: Several studies have been done on tobacco-related problems but the majority of them is not dealing with anxiety disorders or other behavioral changes. Nicotine has been described as the main component of cigarette, which is responsible for a wide variety of neurochemical and behavioral effects. Considering the effect of the cholinergic system on anxiety behavior, the aim of the present study was to: a) evaluate the anxiety behavior, b) determine cholinesterase's (ChE) isoforms (G1, G4) activity in central hemispheres and cerebellum and c) determine the ratio of the activity of two ChE isoforms in the selected brain regions of adult male mice after cigarette smoke exposure.

Methods: Mice were divided into 3 groups: control and those exposed to 1 or 2 cigarettes. The exposure was carried out in a unique smoking device with whole body exposure in cigarette smoke. The anxiety behavior assessed using the open field test by measuring, in a 10 min task, the time mice spent remained close to the walls of the open field apparatus (thigmotaxis time).

Results: The results showed a statistically significant increase in thigmotaxis time only in the group that exposed to smoke of 2 cigarettes. Subsequently, after the mice sacrifices, the central hemispheres and cerebellum were isolated. The activity of G1 and G4 isoforms of ChE was determined, in both salt-soluble and detergent-soluble fraction respectively, by using Ellman's colorimetric method. Also, the ratio of the activity of the two isoforms in the brain regions was evaluated, since this ratio has been associated with the pathophysiology of each tissue.

Conclusions: In conclusion, the results show that the anxiety behavior was affected only after the exposure of the smoke of 2 cigarettes. The cigarettes smoke exposure inhibits with a tissue-specific manner the ChE activity of the two brain regions as well as the G1/G4 ratio of ChE's isoforms activity.

ABSTRACT BOOK

PRECLINICAL EVALUATION

A SIX-MONTH SYSTEMS TOXICOLOGY INHALATION/CESSATION STUDY IN ApoE$^{-/-}$ MICE TO INVESTIGATE CARDIOVASCULAR AND RESPIRATORY EXPOSURE EFFECTS OF A POTENTIAL CANDIDATE AND A CANDIDATE MODIFIED RISK TOBACCO PRODUCT, CHTP 1.2 AND THS 2.2 RESPECTIVELY, COMPARED WITH CIGARETTES

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Background: Smoking is one of the major risk factors in the development and progression of chronic obstructive pulmonary disease and cardiovascular disease. Modified risk tobacco products (MRTP) are being developed to provide substitute products to reduce smoking-related health risks for smokers who are unable or unwilling to quit.

Methods: In a six-month systems toxicology study with ApoE$^{-/-}$ mice, we investigated the impact of cigarette smoke (CS) from the 3R4F reference cigarette, or aerosol from a potential candidate MRTP based on the heat-not-burn principle, the Carbon-Heated Tobacco Product (CHTP) 1.2 and a candidate MRTP, the Tobacco Heating System (THS) 2.2, on the cardiorespiratory system. In addition, we assessed the effect of cessation or switching to CHTP 1.2 after three months of CS exposure. Our systems toxicology approach combined physiology, histology, and multi-omics molecular measurements.

Results: Compared with CS, CHTP 1.2 and THS 2.2 aerosols demonstrated a lower impact on the cardiorespiratory system, including low to absent lung inflammation and emphysematous changes, and reduced atherosclerotic plaque formation. Switching to CHTP 1.2 aerosol and cessation equally resulted in (partial) recovery to Sham-exposed levels. Molecular analyses confirmed lower engagement of pathologically relevant mechanisms by MRTP aerosols than CS. For example, multi-omics analysis of lung revealed complex immuno-regulatory interactions induced by CS and highlighted engagement of the heme–Hmox–bilirubin oxidative stress axis by CS.

Conclusions: Overall, based on apical and molecular analyses, this six-month systems toxicology study highlights a reduced impact of both the potential candidate (CHTP 1.2) and the candidate (THS 2.2) MRTPs on the cardiovascular and respiratory system compared with continued smoking.

ABSTRACT BOOK

THE *IN VITRO* ASSESSMENT OF A TOBACCO HEATING PRODUCT (GLO)

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Background: In recent years there has been an increase in the use and availability of novel and alternative tobacco and nicotine products, including tobacco heating products (THP) and e-cigarettes (electronic nicotine delivery devices (ENDS)). As these products do not burn tobacco (THPs) or contain tobacco (ENDS), the toxicant profile of their aerosols is greatly reduced in comparison to cigarettes, and therefore hold promise as potentially reduced risk products (PRRP). We have published on the use of an integrated assessment framework generating data using pre-clinical, clinical and population studies to assess the reduced risk potential of new products at the individual and population level.

Methods - Results: The challenges and opportunities in applying *in vitro* methods for the assessment of a THP, glo, will be presented, exploring the generation and exposure of test aerosol, evaluating the availability of an *in vitro* toolbox and application to adverse outcome pathways (AOPs). Glo was assessed across a range of *in vitro* regulatory toxicological assays specifically measuring mutagenicity and cytotoxicity, and when compared to conventional cigarettes showed greatly reduced responses. Using high content screening approaches and human-cellular based *in vitro* assays, some of the key events for smoking related diseases such as COPD and CVD could be assessed. Glo demonstrated reduced biological responses across all test systems when compared to conventional cigarettes. Using reconstituted lung epithelial tissue cultures and next-generation sequencing approaches, functional COPD key events were further assessed in response to whole aerosol exposure from glo, and substantial reductions were observed relative to cigarettes. These data supported further AOP development.

Conclusions: Based on these *in vitro* assessments, using an integrated testing approach, glo demonstrated a potential to be reduced risk versus cigarettes. However, a series of clinical and population studies measuring the longer terms effects of these new products on consumers is required to substantiate disease relevant risk reduction.

ABSTRACT BOOK

DEVELOPMENT OF PATHWAY-BASED ASSAYS TO ASSESS CONVENTIONAL AND NOVEL TOBACCO AND NICOTINE PRODUCTS

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Next generation tobacco and nicotine products (NGPs) aim to significantly reduce the harmful impact of cigarette smoking and are gaining consumer acceptability. Rigorous toxicological assessment is essential for ensuring the delivery of safer products to the consumer. Several *in vitro* assessment tools have been developed to monitor toxicological impact of NGPs: cytotoxicity assays (Jaunky et al, 2018), contemporary screening methods (Taylor et al., 2018), *in vitro* toxicological models (Thorne et al., 2018), and gene-based omics data with bioinformatics analysis (Banerjee et al, 2017). A key element of this toxicological assessment is its ability to inform the construction of Adverse Outcome Pathways (AOP), conceptual frameworks that portray existing knowledge between the cause (known as Molecular Initiating Event -MIE), and the effect (known as Adverse Outcome -AO). The toxicology community has started to embrace this initiative and relevant AOPs have been established (Luettich et al., 2017, Lowe et al., 2017).

In this study, we demonstrate the development of a battery of targeted phosphoproteomic assays related to lung pathophysiology that aims to complement gene expression studies and monitor induced alterations of signaling mechanisms (intracellular signaling events) and extracellular activities (cell death, ECM remodeling and cytokine secretion). For this purpose, we developed multiplex targeted assays (termed lung-plex) to monitor alterations in the phosphorylation status of key signaling molecules and alterations in the levels of secreted proteins. As proof of concept, the assays were used to assess the effect of smoke aqueous extracts from the reference cigarette 1R6F on NCI-H292 lung carcinoma cells. Significant changes in stress-related signaling pathways were captured as early as 5 min upon treatment with 1R6F.

Our study demonstrates that the lung-plex multiplex assays can potentially be used for assessing conventional and novel tobacco and nicotine products.

ABSTRACT BOOK

IN VITRO COMPARISON OF THE EFFECTS OF "REDUCED RISK" NICOTINE PRODUCTS AND OF CIGARETTE SMOKE ON ADIPOCYTE SURVIVAL AND DIFFERENTIATION

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Background: Cigarette smoking (CS) has been implicated in cardiovascular, metabolic and respiratory diseases and it constitutes a major cause of lung and other cancers in humans. In the adipose tissue, nicotine has been reported to induce lipolysis, leading to body weight loss[1], while CS has been associated with insulin resistance and hyperinsulinemia[2,3]. Electronic cigarettes (e-cig) and heated tobacco products have been designed to deliver nicotine in a vaping solution or aerosol, without tobacco combustion. Thus, they are assumed to be safer alternatives to conventional cigarettes.

In the present study, we evaluate the cytotoxic effects of CS, e-cig vapor and heated tobacco aerosol, as well as the impact of the contained nicotine on the differentiation of 3T3-L1 pre-adipocytes to "beige" adipocytes.

Methods: By using a commercially available e-cig device and a set of impingers, 327 mg of e-liquid containing 1.2% w/w nicotine and no flavor were evaporated and extracted in 40 mL of culture medium DMEM. Similarly, extracts of three cigarettes and four heated tobacco sticks in DMEM were also produced. LC/MS-MS was applied to determine the levels of nicotine in each extract. Dilutions of these extracts were administered to 3T3-L1 pre-adipocyte cultures and cytotoxicity was measured by the MTT assay. The differentiation of 3T3-L1 pre-adipocytes to beige adipocytes was obtained in the presence of differentiation inducers, as described previously[4]. The cells were exposed to either nicotine (1 or 3 ug/mL) or CS extract dilutions (1% and 10%), throughout the differentiation process. The differentiation efficiency and adipocytic phenotype were assessed by Oil Red O staining and by analyzing the expression of marker genes characteristic of brown adipose tissue by RT-PCR.

Results: The quantification of nicotine in the media extracts showed that CS and heated tobacco extracts contained 110 ug/mL each, while the e-cig extract contained 60 ug/mL of nicotine. No cytotoxic effects of CS extract dilutions below 10% were observed after 24h and 48h of exposure of the 3T3-L1 cells. The effect of the extracts and of nicotine on 3T3-L1 pre-adipocyte differentiation to beige adipocytes is currently being evaluated.

ABSTRACT BOOK

References

1. *An, Z. et al. Nicotine-induced Activation of AMP-activated Protein Kinase Inhibits Fatty Acid Synthase in 3T3L1 Adipocytes. J. Biol. Chem. 282, 26793–26801 (2007).*

2. *Wu, Y. et al. Activation of AMPKα2 in adipocytes is essential for nicotine-induced insulin resistance in vivo. Nat. Med. 21, 373–382 (2015).*

3. *Chiolero, A., Faeh, D., Paccaud, F. & Cornuz, J. Consequences of smoking for body weight, body fat distribution, and insulin resistance. Am. J. Clin. Nutr. 87, 801–809 (2008).*

4. *Zebisch, K., Voigt, V., Wabitsch, M. & Brandsch, M. Protocol for effective differentiation of 3T3-L1 cells to adipocytes. Anal. Biochem. 425, 88–90 (2012).*

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ABSTRACT BOOK

REVERSIBILITY OF *IN VITRO* BIOLOGICAL EFFECTS IN CIGARETTE SMOKE-EXPOSED 3D LUNG TISSUES FOLLOWING SWITCHING TO A TOBACCO HEATING PRODUCT

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Background: Tobacco heated products (THPs) potentially offer a safer alternative to combustible cigarettes. Recent *in vitro* studies have shown reduced biological effects of THPs compared to 3R4F reference cigarette smoke. Existing *in vitro* data, however, generated by performing acute, single exposures, is not reflective of consumer use. Furthermore, the reversibility of the biological effects of cigarette smoke following switching to THPs has not been extensively studied *in vitro*.

Methods: A feasibility study was conducted to assess the potential of using MucilAir™ organotypic tissues in a 4-week repeated exposure study. Tissues were exposed to 3R4F smoke (15 mins, 3 times/week) for 2 weeks, after which the cohort were split into three groups, a further 2-week repeated exposure to 3R4F, a switch to THP or a switch to air. Whole aerosols were generated at the Health Canada Intense smoking regime. Endpoints assessed included cytotoxicity, tight-junction integrity and cytokine expression (33 cytokine panel). The results were compared to a continuous air exposure control at week 4.

Results: During the 4-week repeated exposure, LDH release remained below 10% and TEER above 500 Ω/insert, indicative of tissue integrity. After two weeks 3R4F repeated exposure, an increase in cytokine expression was observed (14 FC>1.5, $p<0.05$), however following 4-week 3R4F repeated exposure, a strong differential cytokine expression was demonstrated, with 14 responsive cytokines including MMP-9, IL-6, IL-4, IL-1α, VEGF ($p<0.05$, FC>1.5). However, tissues that were switched to THP aerosols for 2 weeks following 3R4F repeated exposure, demonstrated lower cytokine expression with only eotaxin-3 and MMP-9 remaining significantly increased ($p<0.05$, FC>1.5).

Conclusions: We have demonstrated the feasibility of repeated aerosol exposure *in vitro* with MucilAir™ tissues remaining viable over the exposure duration. Switching to THP after 2 weeks repeated 3R4F exposure, reversed *in vitro* biological effects, with inflammatory cytokine expression greatly reduced compared to 4-week 3R4F exposure.

ABSTRACT BOOK

SMOKING CESSATION

EFFECTS OF VARENICLINE AND NICOTINE REPLACEMENT THERAPY ON ARTERIAL ELASTICITY, ENDOTHELIAL GLYCOCALYX AND OXIDATIVE STRESS DURING A 3-MONTH SMOKING CESSATION PROGRAM

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Background and aims: The effects of medically-aided smoking cessation on vascular function and oxidative stress are not fully clarified.

Methods: A hundred eighty-eight current smokers were randomized to varenicline or nicotine replacement treatment (NRT) for a 3-month period. We assessed: a) augmentation index (Aix) and pulse wave velocity (PWV); b) perfusion boundary region (PBR) of sublingual microvessel (range: 5-25μm), an index of the endothelial glycocalyx using Sideview, Darkfield imaging; c) the exhaled CO; d) the malondialdehyde (MDA) and protein carbonyls (PC) plasma levels, markers of oxidative stress at baseline, and after 3 and 12 months.

Results: After 3 months of treatment CO, MDA, PC and Aix were decreased in all subjects (median CO: 25 vs 6 ppm, MDA: 0.81 vs. 0.63 nmol/L PC: 0.102, vs 0.093 nmol/mg protein, Aix: 13% vs. 9%, $p<0.05$) while PWV remained unchanged. Endothelial glycocalyx integrity showed a greater improvement in the varenicline than the NRT (PBR range 5-9μm: 1.07±0.02 vs. 1.17±0.02μm, $p=0.03$) in parallel with the greater CO reduction (5 vs 7 ppm, $p=0.02$). At 1 year follow-up, MDA, PC, Aix and PBR at 5-25μm range were further improved in subjects who abstained from smoking (n=84 out of 188) while the above markers and PWV deteriorated in relapsed smokers ($p<0.05$).

Conclusions: A smoking cessation program using varenicline or NRT for 3 months resulted in a decrease of CO, oxidative stress, arterial stiffness and restored endothelial glycocalyx. These effects were more evident after varenicline, likely because of a greater CO reduction, and were maintained after 1 year only in subjects who abstained from smoking.

ABSTRACT BOOK

ROLE OF HEALTHCARE PRACTITIONERS IN ENABLING AND ACCELERATING SMOKING CESSATION AMONG THEIR SMOKER PATIENTS— A UK PERSPECTIVE

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The UK offers a very good example of the most comprehensive policies in smoking cessation giving a wide range of product and service options to smokers for quitting.

Recent findings from surveys among healthcare practitioners (HCPs) such as GPs, pharmacists and dentists highlights a large gap between a progressive tobacco harm reduction policy at the national level and misinformation regarding available smoking cessation options at the practice level among HCPs.

At their peak of performance in 2011, the UK Stop smoking services resulted in over 100,000 quitting successfully that year. However, their contribution in absolute numbers as well as the proportion of smokers quitting successfully with their help has declined to around 40,000 per year[1]. This decline in performance was happening at the same time as funding in stop smoking services was being dramatically cut[2].

The void left by the stop smoking services needs to be urgently filled. With over 60% of smokers in the UK seeing an HCP at least once a year[3], the reach and impact of HCP training cannot be overemphasised. Training of HCPs with the latest guidance on smoking cessation can be very empowering- allowing the HCPs to customise their advice to patients depending on their needs and wants, thus ensuring higher quit and lower relapse rates.

UK's Centre for Health Research and Education (CHRE) has embarked on a national lecture series specifically targeted to HCPs with the sole aim of updating and upskilling them to deliver stop smoking advice confidently to their smoker patients. In parallel, CHRE is validating a modular, digitally enabled toolkit to deliver customised smoking cessation solutions to smokers and their HCPs alike. This is a great opportunity for UK HCPs to convert a world leading policy environment to a smoke-free UK.

References

1. *https://www.frontier-economics.com/media/1113/20171106_working-towards-a-smoke-free-england_frontier.pdf*

2. *http://ash.org.uk/wp-content/uploads/2019/03/2019-LA-Survey-Report.pdf*

3. *https://www.ncsct.co.uk/usr/pub/delivery-and-monitoring-guidance-2011-and-2012.pdf*

ABSTRACT BOOK

E-CIGARETTE USE AND BIOCHEMICALLY CONFIRMED SMOKING STATUS OF A RANDOM SAMPLE OF VAPESHOPS CUSTOMERS IN GREECE

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Background: To understand the public health impact of e-cigarette use it is important to examine the profile of e-cigarette users. The purpose of this study was to evaluate the biochemically-confirmed smoking status and e-cigarette use among customers of a random sample of vapeshops in the greatest area of Athens in Greece.

Methods: Out of a list of 135 vapeshops in Athens, 14 vapeshops were randomly selected for the study. Shops were visited on random days (from Monday to Friday) and hours (morning or afternoon). The only inclusion criterion for participation was the participants being adults (18 years), buying products for own use and willing to participate to the study.

Results: A total of 309 adults participated in the study. The average age of the participants was 36.3 years, with 215 (69.6%) being males. They were using e-cigarettes for a median of 13.1 months. The vast majority (98.0%) reported being daily smokers before e-cigarette use initiation. Regular smokers were 285 (92.2%), while occasional smokers were 18 (5.8%). Only 1.0% reported never smoking while 1% had quit smoking before e-cigarette use initiation. Most participants reported being daily e-cigarette users by (83.5%), were using tobacco flavors, but (58%) of participants reported using at least 1 non-tobacco flavor. Smoking cessation/reduction was the most important reason for initiating e-cigarette use. Biochemically verified smoking cessation (Eco<10ppm) was detected in 70.2-80.6% of participants.

Conclusions: Almost all e-cigarette users, customers of a random sample of vapeshops in Athens, reported being past daily users and the majority were successful in quitting smoking. E-cigarettes probably have a positive Public Health impact.

TRANSITIONS IN ADULTS' CIGARETTE SMOKING STATUS ASSOCIATED WITH USING JUUL VAPING PRODUCTS FOR THREE MONTHS

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Objective: To examine adults' cigarette smoking status at the point of their first purchase of a JUUL e-cigarette, and to examine rates of key transitions in cigarette smoking status over adults' first three months of using JUUL vaping products.

Methods: Participants were 55,414 adults (≥21 years) in the United States recruited at their first purchase of a JUUL Starter Kit in a retail store or online through JUUL's e-commerce platform. Online surveys assessed past 30-day patterns of use of JUUL vaping products, conventional cigarettes and other e-cigarette products, monthly for three months. We report rates of three transitions in smoking status observed at each assessment: (i) current smoker to former smoker (cessation); (ii) former smoker to past 30-day smoker (re-initiation); and (iii) never smoker to past 30-day smoker (initiation).

Results: New JUUL users were current smokers (49.4%), non-current past 30-day smokers (23.2%), former smokers (13.3%) and never smokers (8.9%). Approximately 25.1% of baseline current smokers and 42.3% of baseline non-current past 30-day smokers reported past 30-day smoking abstinence at three months. In contrast, 11.3% of baseline former smokers and 7.7% of baseline never smokers were past 30-day smokers at three months. Daily users of a JUUL e-cigarette and those who did not use a secondary e-cigarette were more likely to have quit smoking and less likely to have re-initiated smoking at three months. Primary users of Mint or Mango flavored JUUL pods were least likely to have initiated smoking at three months.

Conclusions: Using JUUL vaping products for three months was more strongly associated with smoking cessation than with smoking initiation or re-initiation in this cohort of adult new users. If JUUL products are demonstrated to present fewer health risks than cigarette smoking, the observed patterns of JUUL-cigarette transitions would likely yield a short-term net reduction in harm to this cohort.

ABSTRACT BOOK

ASSOCIATION OF FLAVORED NICOTINE SALT POD SYSTEM USE AND SUBSEQUENT SWITCHING BEHAVIOR

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Background: We examined whether: (1) Adult smokers who use nicotine salt pod systems (NSPS) in non-tobacco flavors differ from those who use tobacco-flavored NSPS; and (2) NSPS use in non-tobacco (vs. tobacco) flavors is prospectively associated with past 30-day cigarette smoking abstinence ("switching").

Methods: Adult past 30-day smokers who purchased a JUUL starter kit (N=19,595) completed 30-day, 60-day, 90-day and 180-day follow-up assessments. At the 30-day assessment participants reported the JUUL flavor they used most often in the past 30 days: (1) tobacco flavor; (2) mint/menthol; (3) non-tobacco/mint/menthol flavors (NTM). At the three subsequent assessments participants reported if they had smoked in the past 30-days (yes/no). Preliminary analyses assessed differences in demographic and smoking characteristics by NSPS flavor; repeated-measure logistic regression models analyzed the association between NSPS flavor and switching from smoking to JUUL at the 60-day, 90-day and 180-day assessments.

Results: At the 30-day follow-up, 15.2% of participants primarily used tobacco flavors, 31.3% used mint/menthol and 53.5% used NTM flavors. Participants who used tobacco flavors, compared to mint/menthol and NTM flavors, respectively, were, on average, older (mean age = 35.7 vs. 30.3 vs. 31.5), more likely to be male (59.7% vs. 54.8% vs. 55.1%) and white (85.5% vs. 81.8% vs. 83.9%), smoke more days out of the past 30-days (18.4 vs. 15.8 vs. 16.6), smoke more cigarettes/day (8.5 vs. 6.5 vs. 7.01; ps<0.001). After adjustment for demographic and smoking covariates, use of JUUL in Mint/Menthol (OR [95% CI] = 1.23 [1.11, 1.36]) and NTM flavors (OR [95% CI] = 1.18 [1.08, 1.30]) were significantly associated with switching pooled across follow-up.

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Conclusions: Adult smokers who used JUUL in tobacco flavors significantly differed in their sociodemographic and smoking characteristics from smokers who used JUUL in mint/menthol and NTM flavors. After controlling for these differences, use of JUUL in non-tobacco flavors were associated with higher rates of switching across follow-up period.

ABSTRACT BOOK

TOBACCO HEATING SYSTEM VS COMBUSTIBLE CIGARETTES: CAN LUNG FUNCTION PARAMETERS BE IMPROVED?

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Background: Smoking is one of the major risk factors for the development of respiratory disorders[1]. The Tobacco Heating System (THS) delivers nicotine by heating tobacco (<350°C) to generate an aerosol with reduced levels of toxicants compared with cigarette smoke (CS). After a comprehensive research program, THS has demonstrated potential to reduce the risk of smoking-related diseases; pre-clinical and clinical data associated with respiratory functions are summarized below.

Methods: In an ApoE$^{-/-}$ mouse study, progressive lung function deterioration was assessed by lung histology and measurement of pressure-volume (PV) loop relationships in animals that were switched from exposure to 3R4F reference cigarette smoke to THS aerosol, or cessation, compared with 3R4F CS only. In a randomized, 6-month clinical exposure response study in healthy adult smokers switching to THS compared with continued cigarette smoking (NCT02649556), lung function parameters and other risk markers were evaluated.

Results: In the ApoE$^{-/-}$ mouse study, histopathology indicated reduced accumulation of pigmented lung alveolar macrophages, known to be involved in lung remodeling processes, in THS-exposed mice compared with 3R4F CS-exposed mice. At Month 8, an upward/leftward shift in PV loops from the Sham curve, indicative of emphysematous changes and lung function impairments, was found in 3R4F CS-exposed mice, while minimal effects were detected in animals exposed to THS or switched to cessation. In the exposure response study, the post-bronchodilator percent predicted forced expiratory volume in 1 second was 1.28 higher (95% confidence interval: 0.248, 2.232; $p<0.05$) after 6 months among subjects predominantly using THS compared with those continuing to smoke cigarettes.

Conclusions: Pre-clinical and clinical data showed lower deterioration of lung function over time upon THS switching, while further impairments were observed after continued cigarette smoking. This data is in line with the potential for THS to reduce the risk of harm.

References
1. *U.S. Department Of Health And Human Services. Public Health Service Office of the Surgeon General. Report of the Surgeon General, 2014.*

ABSTRACT BOOK

THE EFFECT OF SMOKING IN PATIENTS WITH PARKINSON'S DISEASE

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This study intends to explore the relationship between cigarette smoke & the incidence of Parkinson's Disease (PD) in patients attending the Melomed PD clinics.

PD, a progressive neurodegenerative disorder, affects movement. The incidence increases with age. Approximately 1% of the population older than 60 years is affected, with 4% diagnosed before the age of 50. It is estimated that 7-10 million people worldwide are living with PD.

Tobacco smoke contains many chemicals, with nicotine being a major component. Nicotine stimulates the nicotinic acetylcholine receptor (nAchR), mimicking the brain chemical acetylcholine. It is well known that acetylcholine has effects on the brain circuitry that is involved in PD. It is currently uncertain whether nicotine alone or in combination with other components of cigarette smoke is protective against PD or not.

What we can infer from the huge body of evidence is that smokers have a 40% lower risk of developing Parkinson's disease in relation to non-smokers. The value of smoking for patients with PD should be further evaluated. The role of following is considered in PD patients – smoking, Nicotine & Reduced Harm alternatives.

Furthermore, a strong inverse association between smokeless tobacco use and PD risk has been reported. A recent meta-analysis of seven cohort studies on snus and tobacco smoking showed that nonsmoking men who used snus had a 60% lower risk of PD compared with never snus users. Results indicated an inverse dose-response relationship between snus users and PD risk suggesting that nicotine or other components of the tobacco leaves may influence the development of PD.

Currently, there's no cure for PD. Medications help manage symptoms. Given the recent introduction of reduced risk products e.g. e-Cigarettes, Heat-not-burn, the outcome should be similar to that seen with the meta-analysis and would add to the current treatment approaches available to the Physician.

ABSTRACT BOOK

TWO FAST GC-MS METHODS FOR MEASUREMENT OF NICOTINE, PROPYLENE GLYCOL, VEGETABLE GLYCOL, ETHYL MALTOL, DIACETYL AND ACETYL PROPIONYL IN REFILL LIQUIDS FOR E-CIGARETTES

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Background: E-cigarettes, an alternative for tobacco cigarettes, are becoming a rising lifestyle trend, even though their safety has not been fully studied. The toxicity of certain ingredients, such as diacetyl and acetylpropionyl, that are potentially present in the refill e-liquids, renders the development of analytical methods for their quantitation a necessary step. The aim of this study is to develop analytical methodologies for the quality analysis of diacetyl and acetyl propionyl and for the quantitative analysis of nicotine, propylene glycol (PG), vegetable glycol (VG) and ethyl maltol.

Methods - Results: Hence in this study, a Trace GC 2000 series coupled with a GC Q plus tandem mass spectrometer and a MS-5 column was used. Two analytical methods were developed. The first method was able to determine simultaneously the PG, the VG and the ethyl maltol, including the 3-methoxyphenethyl alcohol as internal standard, after a derivatization step using the N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) reagent. The second method was used for the quantitative determination of the nicotine using the terbuthylazine as internal standard and the detection of derivatized diacetyl and acetyl propionyl using the o-phenylenediamine reagent in e-liquids. The calibration curves were in the range of 30-100 mg mL^{-1} for PG, 20-100 mg mL^{-1} for VG, 0.1-0.5 mg mL^{-1} for ethyl maltol and 3-20 mg mL^{-1} for nicotine. The detection limit for diacetyl and acetyl propionyl was 5 μg mL^{-1}. The methods were fully validated and they were applied in commercial products.

References
1. *Pisinger, C. & Døssing, M. A systematic review of health effects of electronic cigarettes. Prev. Med. (Baltim). 69, 248–260 (2014).*
2. *Hahn, J. et al. Electronic cigarettes: Overview of chemical composition and exposure estimation. Tob. Induc. Dis. 12, 1–12 (2014).*

ABSTRACT BOOK

TOXICITY CLASSIFICATION OF E-CIGARETTE FLAVORING COMPOUNDS BASED ON EUROPEAN UNION REGULATION: ANALYSIS OF FINDINGS FROM A RECENT STUDY

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Background: A recent study raised concerns about e-cigarette liquids toxicity by reporting the presence of 14 flavouring chemicals with toxicity classification. However, the relevant toxicity classification was not estimated according to the measured concentrations. The purpose of this study was to calculate the toxicity classification for different health hazards for all the flavouring chemicals at the maximum concentrations reported.

Methods: The analysis was based on the European Union Classification Labelling and Packaging regulation. The concentration of each flavouring chemical was compared with the minimum concentration needed to classify it as toxic. Additionally, toxicity classification was examined for a theoretical e-cigarette liquid containing all flavouring chemicals at the maximum concentrations reported.

Results: There was at least one toxicity classification for all the flavouring chemicals, with most prevalent classifications related to skin, oral, eye and respiratory toxicities. One chemical (methyl cyclopentenolone) was found at a maximum concentration 150.7% higher than that needed to be classified as toxic. For the rest, maximum reported concentrations were 71.6% to >99.9% lower than toxicity concentrations. A liquid containing all flavouring compounds at the maximum concentrations would be classified as toxic for one category only due to the presence of methyl cyclopentenolone; a liquid without methyl cyclopentenolone would have 66.7% to >99.9% lower concentrations of flavourings than needed to be classified as toxic.

Conclusions: The vast majority of flavouring compounds in e-cigarette liquids previously reported were present at levels far lower than needed to classify them as toxic. Since exceptions exist, regulatory monitoring of liquid composition is warranted.

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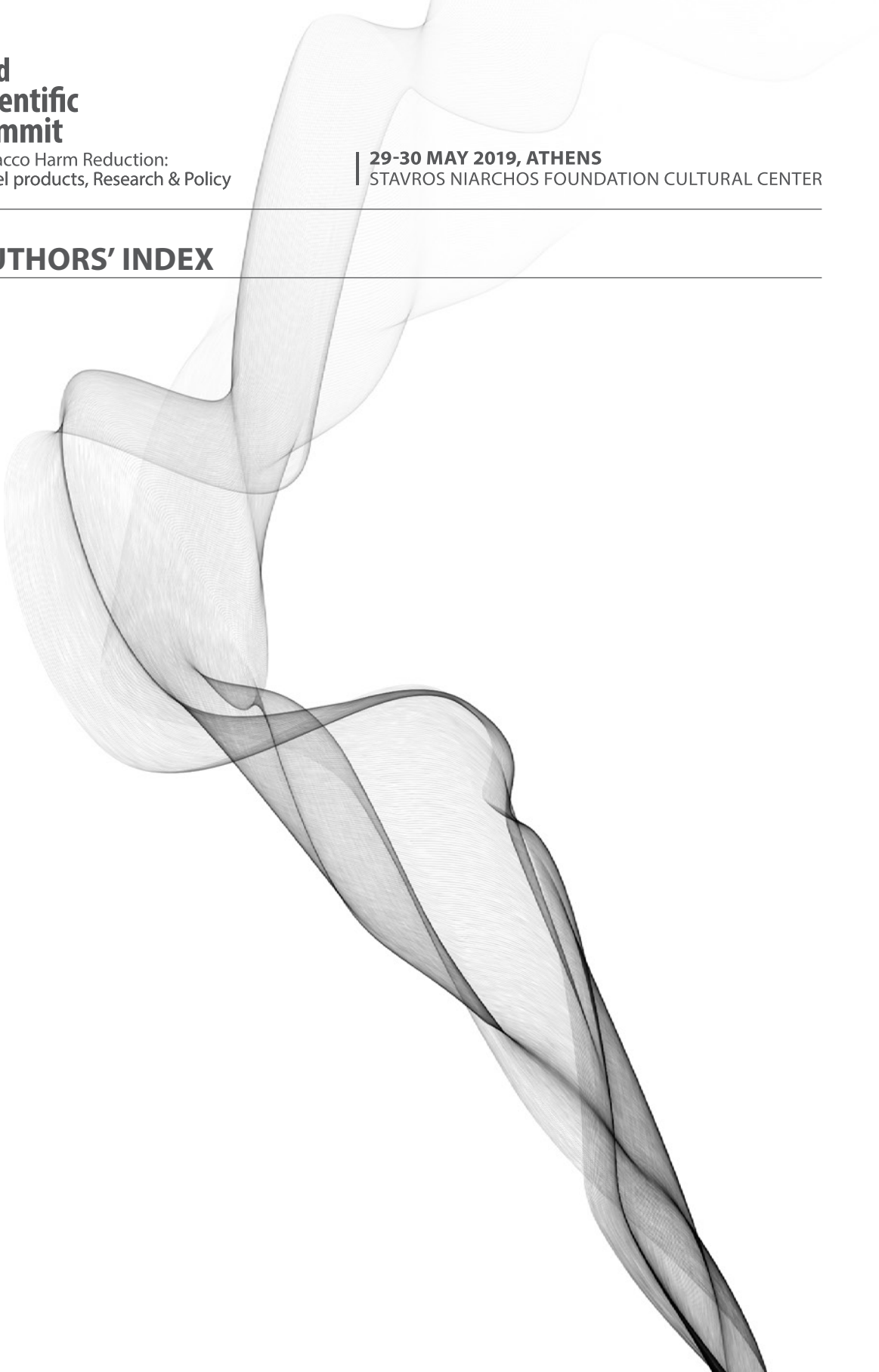

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