1st Scientific Summit

Tobacco Harm Reduction:
Novel products, Research & Policy

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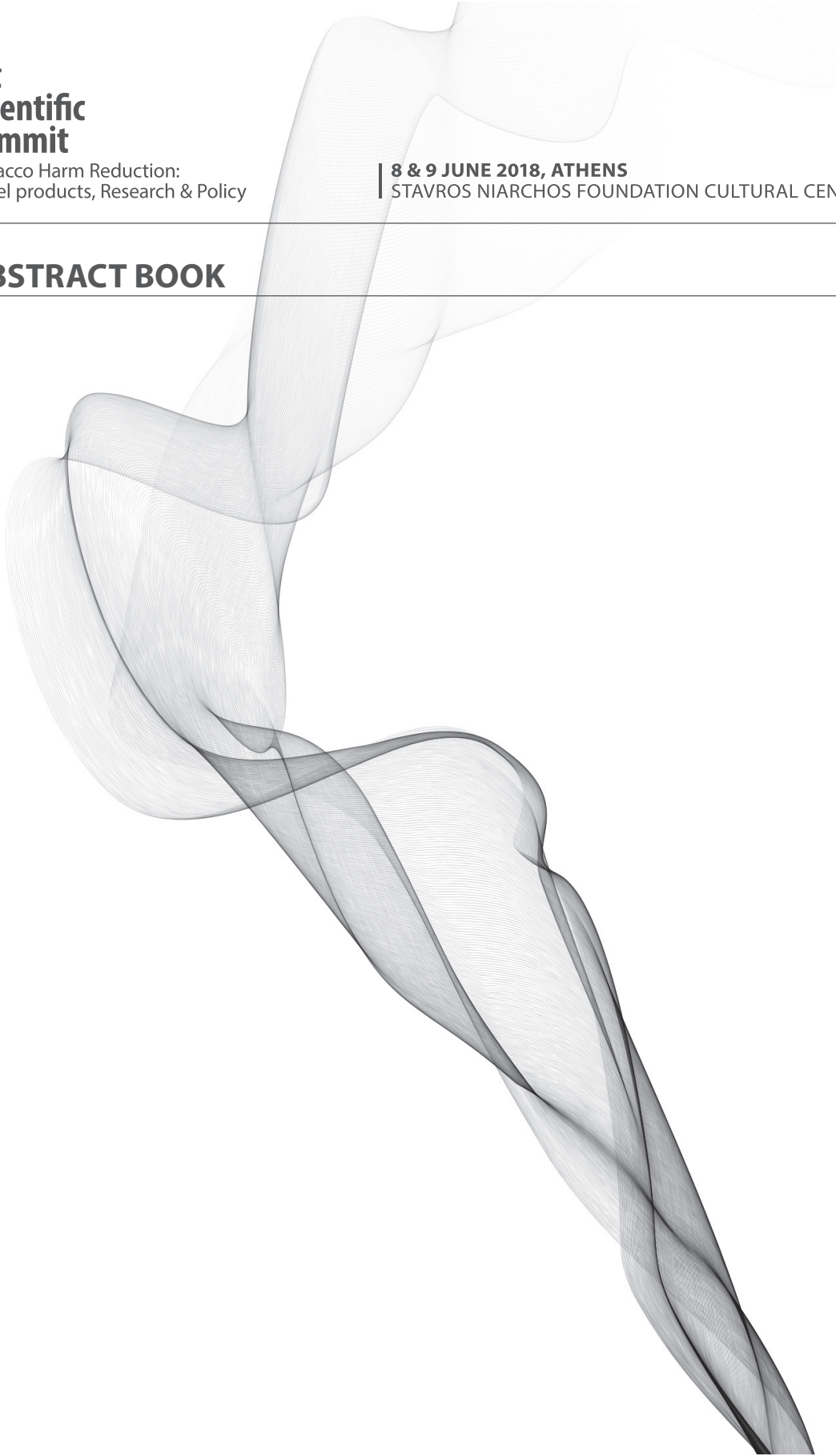
ABSTRACT BOOK



ABSTRACT BOOK

BIOMARKERS' EVALUATION IN ANIMAL OR HUMAN STUDIES

COMPARISON OF THE EFFECTS OF E-CIGARETTE VAPOR WITH CIGARETTE SMOKE ON LUNG FUNCTION AND INFLAMMATION IN MICE

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Electronic cigarettes (e-cig) are advertised as a less harmful smoking cessation tool. We aimed to assess the in vivo effects of e-cigarette vapor in the lung and to compare them to those of cigarette smoke (CS).

We exposed C57BL/6 mice for either 3 days or 4 weeks to ambient air, CS or e-cig vapor containing: i) propylene glycol/vegetable glycerol (1:1; PG:VG), ii) PG:VG with nicotine (G:VG-N), or iii) PG:VG with nicotine and flavor (PG:VG- N+F) and determined oxidative stress, inflammation and pulmonary mechanics.

E-cig vapors increased bronchoalveolar lavage fluid (BALF) cellularity, as well as BALF and lung oxidative markers in most cases comparably to CS; the effects of PG:VG-N+F were evident at both the 3 day and 4 week groups and were most prominent. Unlike CS, no e-cig vapor affected the lung structure. PG:VG-N+F up regulated interleukin-1β and interleukin-6 at 3 days, but not 4 weeks; the reverse was true for CS-exposed animals. After 3 days, PG:VG altered tissue elasticity, static compliance and airway resistance, while after 4 weeks, CS was the only treatment adversely affecting these parameters. Airway hyperresponsiveness in response to methacholine was increased similarly in the CS and PGVG-N+F groups.

Our findings suggest that exposure to e-cig vapor for 3 days triggers inflammatory responses and perturbations in respiratory system mechanics comparable or worse than CS, which, in contrast to CS, tend to became milder at 4 weeks of exposure. The added flavor in e-cigs negatively impacts both lung inflammation, as well as respiratory function.

This study was funded in part by a grant by Nobacco and Alterego, vendors of e-cigarettes.

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 over-activation 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: Thirty current smokers (mean age 48 years±5) without cardiovascular disease were randomized to smoke either a conventional 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, and 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).

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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.

N=30	baseline	Conv-cig	e-cig	p-value
LTA-ADP %	64.0±16	68±20	76±7	<0.05
EPI %	62±14	64.75±16	74.8±8	<0.05
COLL %	14.9±7	16.7±15.4	44.8±15.9	<0.05
PFA-100 sec	123.20±66	144.50±38.9	125.20±16.0	<0.05
MDA μmol/L	1.22±0.1	1.43±0.1	1.09±0.1	<0.05
Aix %	29.5±2	30.5±2	25.6±2	<0.01

Effects of electronic or conventional cigarette smoking on platelet function during one month of use

ABSTRACT BOOK

IS VARENICLINE ADMINISTRATION FOR SMOKING CESSATION ASSOCIATED WITH SLEEP DISTURBANCES? A POLYSOMNOGRAPHIC EVALUATION

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Recently, several smoking-cessation interventions are being tested. Among-them varenicline seems to be a robust one in terms of abstinence rates. However, its prescription may be accompanied by side effects during sleep such as vivid dreaming, increased arousal and poor sleep quality. Although these findings have been reported, they have not been quantified until now through an objective manner.

This study is part of the ongoing H2020-funded EU SmokeFreeBrain project, which addresses the effectiveness of several antismoking approaches at vulnerable groups of smokers like patients suffering from asthma or from chronic obstructive pulmonary disease. A secondary aim of the project is to quantify the side effects of varenicline during sleep.

The quantification is being performed through an elaborate computational framework which integrates various analyses performed of polysomnographic (PSG) data such as spectral analysis and functional connectivity on electroencephalographic (EEG) data, time-frequency analysis of heart-rate variability and physiological network based graph metrics.

Early results were obtained from 17 patients who performed two overnight PSG recordings (before and 21 days after varenicline treatment). Preliminary findings from a small number of data epochs indicate the co-existence of facilitated sleep onset probably due to smoking cessation but poorer sleep quality afterwards. The first is mainly due to increased alpha activity prior to sleep onset and is located on frontal regions. However, there is concrete evidence of increased arousal during the entire non-rapid eye movement (NREM) sleep. Its EEG sign is the increased beta band functional connectivity.

These findings should be further validated by integrating features from the aforementioned recording modalities and with the macro-architecture derived from manual sleep scoring according to the guidelines of the American Association of Sleep Medicine in order to provide a clearer view of varenicline's impact on sleep.

ABSTRACT BOOK

MONITORING THE TRANSITION FROM CIGARETTE SMOKING TO E-CIGARETTE USE: THE SMOKEFREEBRAIN STUDY

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Electronic cigarettes (EC) have proven very popular with smokers and a meteoric rise in their usage is currently under way. Nonetheless there is still ongoing uncertainly over their safety and impact on health.

Aims and Objectives: This project aims to monitor human volunteers as they switch from heavy (>10 cigarette/day) smokers to EC-only use. Monitoring of markers comprise expired carbon monoxide, DNA methylation, oxytocin, cortisol, nicotine, cotinine and tobacco-specific nitrosamines (NNK, NNAL, NNAL) levels in urine and saliva together with psychometric data, heart rate and blood pressure. In addition electroencephalography (EEG) was used to determine changes in brain activity following transition to EC use.

Methods: Volunteers were recruited at St. George's University of London. Volunteers were allowed to select their own EC and e-fluid. Blood, saliva, urine, heart rate, blood pressure, expired CO and questionnaire data (nicotine dependence, withdrawal, anxiety, depression, mood, quality of life and sleep quality) were collected throughout the 28-day trial (days1,2,3,21,28) and EEG resting data (eyes open-eyes fixate-eyes closed, each 3 x 30s) was collected prior to smoking cessation and on day 21. Nicotine, cotinine, NNK, NNAL and NNN were determined by UHPLC-HESI-HRMS. Compliance was monitored via exhaled CO and urinary anabasine.

Results: Urinary nicotine and cotinine and nitrosamines decrease significantly following transition to EC and behavioural data have revealed modest reductions in nicotine craving, nicotine withdrawal symptoms, heart rate and improvement in sleep quality following the transition to EC. Preliminary analysis of the EEG data identified common statistically significant activity increases in the left insula in the delta band and the anterior cingulate cortex in the theta and alpha bands between pre- and post- transition to EC-only use. 28% of participants relapse during the month following transition to e-cigarettes.

Conclusions: Switching to EC quickly induces beneficial changes in exposures to toxicants, and psychometrics.

Funding: This work is supported by the ongoing grant SmokeFreeBrain, agreement number 681120 under H2020 funding from the European Union and by a PHE-funding PhD studentship.

ABSTRACT BOOK

CARDIOVASCULAR EFFECTS OBSERVED WHEN USING THE TOBACCO HEATING SYSTEM (THS) COMPARED WITH CONTINUED SMOKING

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Cigarette smoking is causally linked to the development of cardiovascular disease (CVD), contributing to endothelial injury and dysfunction, a proatherogenic lipid profile, chronic inflammation, and an abnormally increased tendency toward coagulation. Evidence from *in vitro, in vivo,* and population-based studies led to the recognition that vascular inflammation and oxidative stress are mechanisms leading to atherogenesis and CVD. Cigarette smoke (CS) contains +6,000 chemicals, 93 of which are categorized as harmful and potentially harmful constituents (HPHC) by the U.S. Food and Drug Administration. Twelve HPHCs have been identified as cardiotoxic. HPHCs, oxidants, and carbon-based nanoparticles (cbNPs) contained in CS are mediators of endothelial dysfunction and other pathological mechanisms underlying atherosclerosis and CVD.

To reduce the risk of CVD and other smoking-related diseases, Philip Morris International (PMI) has developed Reduced Risk Products, such as the Tobacco Heating System (THS), that heat instead of burning tobacco, thereby generating an aerosol containing no cbNPs and significantly reduced levels of HPHCs vs. CS. The aim of PMI's assessment program is to demonstrate that switching to THS has the potential to reduce the risk of smoking-related diseases vs. continued smoking. PMI's assessment program includes in *vitro/in vivo* toxicology testing methods that follow Organisation for Economic Co-operation and Development guidelines and Good Laboratory Practice, a systems toxicology approach, and randomized, controlled clinical studies following Good Clinical Practice.

The results of the THS translational assessment program demonstrate that:

- Cardiovascular toxicants are reduced by >92% in THS aerosol vs. CS.
- THS aerosol does not contain cbNPs.
- The effects of THS aerosol on the adhesion of monocytic cells to

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human coronary endothelial cells *in vitro* are significantly reduced.

■ Switching to THS halted the progression of CS-induced atherosclerotic changes. THS aerosol alone had minimal adverse effects in two ApoE$^{-/-}$ mouse studies over durations of six and eight months.

■ Clinical risk endpoints linked to smoking-related disease (e.g., HDL-C, markers of inflammation, and oxidative stress) are currently analyzed following a six-month randomized, controlled clinical study on THS.

The evidence available to date indicates that switching to THS has the potential to reduce the risk of smoking-related diseases, such as CVD.

ABSTRACT BOOK

COMPARATIVE STUDY OF TOBACCO CESSATION SERVICES IN TWO URBAN SETTINGS: BRAC EXPERIENCE

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According to World Health Organization (WHO), smoking and passive smoking are collectively the biggest preventable cause of death in Bangladesh, with major public health burden of morbidity, disability, and mortality and community costs. Bangladesh is also one of the top ten countries in the world with the highest current smoking prevalence. Smoking or use of tobacco use also increases the risk of developing tuberculosis. According to WHO Global TB Report, 2017, Bangladesh has 223,921 tuberculosis patients out of which 22% smoke.

It is important to evaluate the existing cessation services in Bangladesh and come up with effective interventions helping TB patients to quit smoking. At present, the cessation services for TB patients in Bangladesh are minimal and largely depend upon counselling without any nicotine replacement therapy leading to low quit rate.

For this study, we have compared the tobacco data of quarter 4, 2017 for BRAC supported Khulna and Sylhet urban areas. The number of DOTS centers in Khulna urban area is 2 and the population coverage is 60,384. The number of total patients enrolled in quarter 4 was 56 and 39% of them are male. Among enrolled patients, 30% was smoker (male 82%) and 25% of these smokers smoke cigarette. The percentage of smokeless tobacco users is 25%.

On the other hand, the number of DOTS centers in Sylhet urban is 6 and the population coverage is 438,636. The number of total patients enrolled in quarter 4 was 456 and 57% of them are male. Among enrolled patients, 37% was smoker (male 99%) and 36% of these smokers smoke cigarette. The percentage of smokeless tobacco users is 36%. A detail study in future will help us to gain more significant insights regarding TB patient and smoking.

ABSTRACT BOOK

CONVENTIONAL SMOKING, VAPING AND DRY EYE: A PUBLIC INCIDENCE AND AWARENESS STUDY IN GREECE

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Smoking is an established leading preventable cause of morbidity and mortality. Electronic cigarette vaping is a recently introduced option that claims to provide safer smoking habits to smokers. Smoking is also associated with eye diseases and occasionally dry eye, albeit less popularly established.

The aim of this study was to evaluate the Greek public incidence and awareness on smoking/vaping and dry eye or other eye diseases.

In a pilot study, 500 smokers (250 conventional smokers and 250 vapers), males and females from all age groups and all educational levels and occupational areas, were randomly approached and asked to complete a specially modified questionnaire. Amongst others, the health and eye symptomatology were investigated, as well as the smoking habits, the knowledge of smoking-related diseases, and the attitude against associations between smoking/vaping and dry eye or other eye diseases. Statistical analysis was performed using Chi Square tests. Approximately 95% of the subjects found extremely significant the association of conventional cigarette smoking and health problems such as lung cancer but were quasi aware on the smoking-related eye problems rating the association between blindness and smoking very significant by 68%, or that of dry eye less significant by 80%.

Vaping is believed to be a healthier option of smoking in the vast majority of smokers (90%) and vapers (70%), but not such a favorable factor towards quitting smoking as only approximately 40% of smokers and 50% of vapers believe that e-cigarettes help towards quitting smoking overall. The present study suggests a decreased Greek public awareness on smoking-related dry eye or other eye health problems. Further informing the public is necessary about the various dangers of smoking in smoking-cessation campaigns and public health education.

ABSTRACT BOOK

COMPARATIVE HEALTH STUDY BETWEEN SMOKERS, VAPERS AND EX-SMOKERS IN ACHAIA PREFECTURE, GREECE

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Smoking has always been very addictive for every age and social group. Smoking harms human health as it causes at least 25 life-threatening diseases, such as asthma, lung cancer, strokes, heart diseases and diabetes, and it affects sexual life and reproduction. It is a serious cause of morbidity and mortality and kills 6 million people per year. Electronic cigarettes (ECs) and Heat Non-Burn (HNB) products (e.g. iQOS) are smoke-free alternatives and can act as a stopover towards smoking. They are up to 95% less harmful than conventional cigarette and less addictive. The ECs and iQOS became popular in smokers, because they are closer to smoking than any other nicotine drug and have the same behavior.

A study of a representative sample of approximately 100 smokers living in Achaia prefecture (Greece) was carried out through questionnaires. The aim of this study was to examine their epidemiological characteristics in relation to their smoking/vaping habits. Measurements of arterial pressure, saturation of blood, exhaled CO2 and spirometric control were performed and the health outcomes among these individuals were evaluated.

ABSTRACT BOOK

ADOLESCENT ATTITUDES TOWARDS SMOKING: PSYCHOLOGICAL AND PUBLIC HEALTH ASPECTS

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Despite the implementation of anti-smoking policies in most countries of the developed world, smoking remains a significant public health issue for the adolescent population worldwide. 10-15% of the adolescents identify as smokers. Furthermore, various studies confirm that the majority of adult smokers (60-90%) started smoking before the age of eighteen. Nowadays more and more adolescents are using the e-cigarette, either as a sequence of the conventional cigarette or they begin smoking e-cigarettes right from the start. There are many diverse factors that contribute to the initiation of smoking in adolescence, such as peer pressure, the "feel good syndrome", curiosity and the power of role models.

On the one hand, adolescents will always seek peer approval and acceptance, while on the other hand their friends who smoke will constantly encourage them to smoke. Adolescents report robust beliefs that smoking makes them more popular, attractive or strong; neglecting the possible negative effects of smoking on health. Parental smoking has been long identified as a potential risk factor for adolescent smoking whereas parental disapproval of smoking leading to anti-smoking behavior.

The psychological profiles of teenagers who are most at risk of developing addictive behaviors include characteristics such as low self-confidence, learning disabilities, school failure, tendency to be alone, belonging to minority groups or physical disabilities. Teenagers whose parents have been divorced and have experienced intense fights between their parents - a situation that could lead to unsatisfactory bond between the child and either of their parents - are more vulnerable to addictions.

Addiction is always a case of self-infliction with adolescents, attacking their own bodies, the things they have achieved or their mental resources and thus limiting themselves from their own potential, especially the part that they have previously appreciated most and had worked hard to achieve.

ABSTRACT BOOK

E-CIGARETTE USE AMONG ADOLESCENTS: CONTROVERSIES AND CHALLENGES FOR FUTURE RESEARCH

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As e-cigarette use increases rapidly it is important to examine its appeal, influence and effects among adolescents. The majority of researches indicate that adolescents who use e-cigarettes are susceptible to initiate and continue using other types of cigarettes, tobacco and even cannabis. The e-cigarette has been promoted in multiple ways as a healthier alternative to smoking by the media and even researches that present its benefits in comparison to conventional cigarettes. Contrariwise, numerous studies appear showcasing the perceived harmful cognitions, negative neuropsychological, biological effects of e-cigarettes due to chemical substances that some of their products contain and even the sociological and emotional effects on adolescents. In 2012 the United States Centre of Disease Control and Prevention estimated that e-cigarette experimentation and recent use doubled among middle and high school students, while the 1/10 of them had never used conventional cigarettes. In Greece, significant changes in smoking behaviors have been observed during the past few years, possibly as a combined result of the implemented tobacco control and austerity measures. A study about smoking, alcohol and drug use among Greek adolescents reported that in 2015 39.2% of 16 year-old high school pupils nationwide had smoked cigarettes at least ones in their lifetime, while the 13% of whom were regular smokers, predominantly males (Kokkevi et al. 2016). Another study reported that half of the population of 15 year-old pupils in Greece who have used combustible cigarettes have also tried e-cigarettes (Fotiou et al. 2015).

Combining these findings, we discuss the use of e-cigarette during adolescence, the probable reasons they initiate and continue it, its physical, emotional and social effects. In conclusion, stricter sales regulations (age, product restrictions), public monitoring and health awareness programs could help bring this issue of import to the forefront, while helping adolescents acquire knowledge and adjust their smoking behavior.

ABSTRACT BOOK

PREVALENCE OF TOBACCO USE AMONG TB PATIENTS AND IMPROVING TB TREATMENT OUTCOMES BY REDUCING TOBACCO CESSATION

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Background and implementation: Tobacco consumption through inhalation has increased substantially especially in developing countries like Bangladesh. Since 2011, BRAC, a non-government organization, has incorporated smoking cessation intervention into Directly Observed Therapy (DOT) programs in all urban settings. Such Intervention was necessary because smoking tobacco is associated with the development of tuberculosis (TB) and decreases the effectiveness of TB treatment.

Intervention: BRAC supported urban TB centres of all division covering 7.8 million populations were selected for intervention. TB programme staff was trained on tobacco control with particular focus on harmfulness of smoking, smokeless tobacco, second-hand smoking and its impact on TB. All the tools were based on Union Smoking Cessation guideline and adopted in Bengali. Counselling is given to patients for smoking cessation during initiation of treatment and subsequent visits to TB centre. A brief counselling is done by Shasthya Shebika (Frontline Community Health Worker) during DOT.

Results and lessons learned: In 2017, a total of 20843 TB patients were enrolled. Among them, 22% were smokers of which 98% male and 24% patients were exposed inside home, of them 57% female. 6% patients were smokeless tobacco; of them 62% are female. At the end of treatment 73% quitted smoking and treatment success rate were 94% in intervention areas.

Conclusions and key recommendations: Tobacco cessation among TB patients is found to be more effective and improving treatment results as well as enrich programme performance.

ABSTRACT BOOK

TOBACCO SMOKING, ELECTRONIC CIGARETTES AND OCULAR DISEASES

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It is known that tobacco smoke is a complex mixture containing multiple chemicals, such as carbon monoxide, acetaldehyde and formaldehyde. According to WHO, tobacco smoking is responsible for 5.4 million deaths worldwide. Amongst the most common tobacco smoke related causes of death are cardiovascular disease and various types of cancer. Additionally, smoking is a risk factor for many eye diseases, such as glaucoma, Graves' disease, cataract and dry eye. Dry eye syndrome or keratoconjunctivitis sicca (KCS) is a common dysfunction of tear film, during which there is low eye lubrication due to tear deficiency or excessive tear evaporation. This disorder is characterized by inflammation of the ocular surface and lacrimal glands. The most common clinical symptoms are ocular burning, foreign body sensation and stinging sensation.

An important risk factor for dry eye is smoking: research has shown that smoking can cause a significant reduction of tear film break up time and can lower basal tear secretion and corneal sensitivity, due to the huge number of toxic compounds that the tobacco contains. Also, lipid layer changes that lead to failure of lipid spread and therefore significant increase in tear evaporation rate and decrease in tear film stability have been observed. Generally, the tobacco compounds react with the immune cells and the endothelial cells of conjunctival mucosa, at molecular and cellular level and result in dry eye syndrome.

ABSTRACT BOOK

PREVALENCE OF SMOKING AND ELECTRONIC CIGARETTE USE IN GREECE: A SURVEY OF A REPRESENTATIVE POPULATION SAMPLE IN ATTICA

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One of the main determinants of the public health impact of electronic cigarettes (EC) is to examine prevalence of use in the population and the smoking status of EC users. The purpose of the study was to estimate the prevalence of smoking and EC use in Greece in 2017 in a representative sample of adults in Attica prefecture (3.1 million adult population).

A cross-sectional survey of a representative sample of 4058 adults living in Attica prefecture (35% of the Greek adult population) was performed in May 2017 through computer-assisted telephone interviews. Prevalence and frequency of e-cigarette use were assessed according to the smoking status, and logistic regression analysis was performed to identify correlates of use.

Current smoking was reported by 32.6% of participants while 29.7% were former smokers. Ever e-cigarette use was reported by 54.1% of current smokers, 24.1% of former smokers and 6.5% of never smokers. Past experimentation was the most prevalent pattern of e-cigarette use among ever users ($P < 0.001$), while current e-cigarette use was reported by 5.0% of participants. Almost 80% of ever and 90% of current e-cigarette users were using nicotine. Extrapolated to the whole Attica population (3.1 million), there were 1 million current smokers, 848,000 ever e-cigarette users and 155,000 current e-cigarette users. The majority of current e-cigarette users (62.2%) were former smokers. Only 0.2% of never smokers were current e-cigarette users. Approximately 5% of participants considered e-cigarettes a lot less harmful than smoking. Being current or former smoker were the strongest correlates current e-cigarette use (OR 30.82, 95%CI 10. 21-69.33 and OR 69.33, 95%CI 23.12-207.90 respectively). E-cigarettes were the most popular smoking cessation aid among smokers who have tried to quit in the past 3 years.

E-cigarette use in Greece is largely confined to current or former smokers, Current use and nicotine use by never smokers is extremely rare. The majority of current e-cigarette users were former smokers. Most participants overestimate the harmfulness of e-cigarettes relative to smoking.

ABSTRACT BOOK

RISK PERCEPTION AMONG SMOKERS: E-CIGARETTE AND SMOKING RELATIVE HARM

Brendan Noggle, Maria Gogova, Jessica Rosner, and Ryan Black

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A congressionally mandated report from the National Academies of Sciences, Engineering, and Medicine (NASEM) on the health effects of e-cigarettes suggests that while not without health risks, e-cigarettes are likely far less harmful than conventional cigarettes. We investigated if consumer perception of e-cigarette and cigarette risk is consistent with the NASEM conclusion.

Two waves of the US-representative longitudinal Population Assessment of Tobacco and Health (PATH) study were used to evaluate whether adult smokers and e-cigarette users, including FDA defined vulnerable populations (VPs), misperceive e-cigarette use as equal to or more harmful than smoking cigarettes. FDA defines VPs as persons with low socio-economic status (SES), minority race/ethnicity, LGBTQ sexual orientation, or fair/poor mental health. Logistic regression was used to determine the odds of risk misperception among study groups at each wave after adjusting for age, gender, and education. Tests of differences in the percent of risk misperceptions across waves were performed among adult smokers and VPs who smoke. All analyses used population weights.

Wave 1 odds of misperception were significantly higher among adult smokers relative to e-cigarette users in each study group (all adult smokers OR=5.8, low SES OR=5.4, minority OR=3.0, LGBTQ OR=4.6, mental health OR=3.2, ps≤0.0001). Similar odds were observed in wave 2 as wave 1. The percent of smokers and VP smokers reporting the risk misperception increased across waves (all adult smokers: 44.9% to 59.0%, low SES: 48.1% to 62.4%, minority: 50.0% to 64.4%, LGBTQ: 47.4% to 60.6%, mental health: 44.6% to 59.6%, p_s<0.0001).

Our findings show there are growing misperceptions among adult smokers that e-cigarette use is equal to or more harmful than smoking, a belief inconsistent with the NASEM conclusion. It is critical to provide adult smokers, including those identified as vulnerable by the FDA, with truthful and accurate information about relative risks of tobacco products to advance tobacco harm reduction.

ABSTRACT BOOK

DOES BIOFEEDBACK TRAINING PROMOTE NEUROPLASTIC CHANGES IN SMOKERS? A PRELIMINARY STUDY

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Many studies have explored the linkage between smoking and stress, as stress constitutes an aggravating factor that is present in every phase of nicotine addiction. Moreover, smoking and stress have shown additive effect in various physiologic responses and both of them have been shown to affect the brain function. Smoking affects the organization of resting-state networks, including the default mode network (DMN), in the context of an overall negative impact on central nervous system's functional integrity while prolonged stress may also result in enhancement of DMN activation.

We have focused on biofeedback as a possible promoter of neuroplasticity on the smoking brain, since it has been already demonstrated as a fruitful intervention for stress-related conditions and their physiological manifestations.

A cohort of twenty-seven smokers performed five sessions of skin temperature training and preliminary analysis was performed on resting-state electroencephalographic data, along with clinical and behavioral changes. Functional networks were investigated between cortical sources of the DMN using network-based statistics and graph analysis.

Although the degree of nicotine dependence and the presence of psychiatric symptomatology were significantly improved, clinical status remained stable. Regarding networks, the right temporal pole cortex and the right ventrolateral prefrontal cortex marginally increased their output towards other nodes, even though network organization and properties were not affected by the biofeedback training regimen. We aim to further explore possible induction of neuroplasticity through neurofeedback and prolonged training.

ABSTRACT BOOK

PRECLINICAL EVALUATION

SIX-MONTH SYSTEMS TOXICOLOGY INHALATION/CESSATION STUDY IN APOE$^{-/-}$ MICE TO INVESTIGATE CARDIOVASCULAR AND RESPIRATORY EXPOSURE EFFECTS OF TWO REDUCED RISK PRODUCTS COMPARED WITH CONVENTIONAL CIGARETTES

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Objectives: Cigarette smoking causes adverse health effects that may occur shortly after smoking initiation and lead to the development of cardiovascular disease, respiratory disease (chronic obstructive pulmonary disease), and various cancers. To reduce the risk of smoking-related diseases, Philip Morris International is developing Reduced Risk Products (RRP) to which adult smokers can switch instead of continuing to smoke cigarettes.

Methods: Engaging a systems toxicology approach combining physiological, histological, and omics endpoints, the effects of a six-month exposure to cigarette smoke (CS) or to aerosols from two RRPs, the Carbon Heated Tobacco Product (CHTP) and the Tobacco Heating System (THS), were investigated in ApoE$^{-/-}$ mice. In addition, the impact of cessation or switching to CHTP aerosol exposure after three months of CS exposure was evaluated.

Results: Our results demonstrated that exposure to CS at a concentration of 28.0 µg nicotine/L causes adverse effects on the lungs, including increased lung volume, lung inflammation, aortic plaque formation, and a dysregulation of the heart transcriptome. In contrast, exposure to either THS or CHTP aerosol at matched nicotine concentrations did not induce lung inflammation or enhance plaque development. Cessation or switching to CHTP aerosol exposure reversed lung inflammatory responses and halted progression of aortic plaques. Transcriptomics analysis revealed that multiple biological pathways were impacted significantly in heart tissue by CS exposure but not by exposure to CHTP or THS aerosols. Both cessation and switching to CHTP aerosol reduced these perturbations to levels similar to those in sham-exposed animals.

Conclusion: In conclusion, in this ApoE$^{-/-}$ mouse study, exposure to aerosol from either THS or CHTP had minimal adverse respiratory and cardiovascular effects. In addition, cessation or switching to CHTP aerosol exposure delayed the progression of CS-induced atherosclerotic and lung emphysematous changes.

ABSTRACT BOOK

ASSESSMENT OF THE glo™ TOBACCO HEATING PRODUCT: TRADITIONAL AND 21ST CENTURY TOXICOLOGY APPROACHES

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Background: Cigarette smoking is a risk factor for many diseases including cardiovascular disease, lung disease, and cancer. Recently there has been an increase in the development and consumer acceptance of novel nicotine and tobacco products including tobacco-heating products (THPs) and vapour products such as e-cigarettes.

Objective: Using a number of *in vitro t*est methods, recently outlined as part of a framework to substantiate the risk reduction potential of novel tobacco and nicotine products, we have assessed the toxicological and biological effects of a tobacco heating product, glo, designed to reduce toxicant exposures. Responses were compared to a 3R4F reference cigarette.

Methods and Results: Exposure matrices assessed included total particulate matter (TPM), whole aerosol, and aqueous aerosol extracts obtained after machine-puffing using the Health Canada Intense smoking regime. Disease relevant endpoints assessed included mutagenicity (Ames and Mouse Lymphoma assays), cytotoxicity (neutral red uptake), tumour promotion (Bhas cell transformation) and endothelial cell migration (wound healing). Contemporary screening approaches employing a luciferase-based reporter gene assay and multiparametric analysis incorporating 10 different toxicity and oxidative stress endpoints were used to assess TPM from products. The tobacco heating product had little or no activity across all the *in vitro* assays assessing when compared to a 3R4F reference product.

Conclusion: These *in vitro* assays have enabled the biological assessment of a tobacco heating product, and results suggest the product shows the potential to reduce health risks. Further pre-clinical and clinical assessments are required to understand further the risk reduction of these novel products at individual and population levels.

ABSTRACT BOOK

SYSTEMS TOXICOLOGY ASSESSMENT OF FLAVORS COMPOUNDS PRESENT IN E-VAPOR PRODUCTS USING HUMAN PRIMARY BRONCHIAL EPITHELIAL CELLS

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Assessing the toxicity of e-cigarettes is challenging considering the large variety of flavored e-liquid mixtures and devices commercially available and the lack of standardized testing methods. While some flavors used in e-liquids are on the FDA's "generally considered as safe" list when ingested or applied topically, there is limited data about their toxicity when they are inhaled.

We recently developed a high-throughput approach to assess the biological impact of e-liquid ingredients on primary human lung epithelial cells *in vitro* (using an advanced cellular assay platform). Submerged cells were exposed to serial dilutions of various single flavor compounds in a solution composed of propylene glycol (41%), vegetable glycerin (38%), and nicotine (0.6%). First, real-time cell viability was assessed over a 24h exposure period by impedance-based measurement. Selected flavors, based on their additional contribution to matrix toxicity, were then further evaluated using a battery of high content screening endpoints. For example, two flavors widely used in e-vapor products (diacetyl and cinnamaldehyde) showed a very similar toxic profile at doses ranging from 0.2 to 0.5% and 0.04 to 0.01% w/w respectively. They appeared to exert their effect mainly at mitochondrial level as demonstrated by a consistent decrease of both mitochondrial membrane potential and mass after 24h of exposure. As a consequence of the mitochondrial damage, oxidative stress signs were also observed. Increased production of reactive oxygen species (ROS), further leading to a consistent cellular antioxidant glutathione (GSH) content depletion, was in fact observed after both 4h and 24h exposure. Increased phosphorylation of histone 2A variant X, hallmark of DNA double strand breaks, was also detected at 24h possibly as a consequence of the increased ROS level. The results of this study will be finally complemented with a microarray-based transcriptomics analysis followed by a computational approach leveraging mechanistic network models to identify and quantify biological perturbations.

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In conclusion, this approach could contribute to classify the inhalation toxicity level of flavors ingredients usually found in e-liquid mixtures and to establish a list of flavor ingredients that can be used in e-cigarettes. This assessment method is aligned with mechanism-based toxicity testing and risk assessment for the 21st century.

ABSTRACT BOOK

IN VIVO QUANTIFICATION AND PHARMACOKINETIC STUDIES OF COTININE IN MICE BY LC-MS/MS

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Nicotine is the primary ingredient of tobacco that is responsible for the addictive properties of smoking in human. Following nicotine uptake in the body, nicotine is metabolized mainly via the P450 enzyme system to primary metabolites. Moreover, nicotine is metabolized by the liver, lungs and kidney and has a biological half-life of 2 hours. Cotinine is the primary metabolite of nicotine, the major alkaloid of tobacco and the active ingredient in cigarettes.

The objective of this study is to develop a sensitive, selective and reproducible LC-MS/MS method for the determination of cotinine in mouse plasma after smoking vaping exposure.

The analytical method that was developed for the detection of cotinine in mouse plasma showed high sensitivity with lower limit of quantification of 0.075 ng/mL and lower Limit of detection of 0.025 ng/mL. Mice were exposed to the smoke of commercially available 0.5, 1.0 and 1.5 cigarettes, the cotinine levels were measured in the plasma and the method had been successfully applied to pharmacokinetic studies.

Following smoke exposure of 1.5 cigarettes, it was found that cotinine had an elimination half-life of 76.42 minutes, a C_{max} of 13.30 ± 0.02 ng/mL, while the apparent volume of distribution Vz/F was 55419.54 mL and the clearance CL/F was 502.64 mL/min.

The quantification of the concentration of cotinine in mouse plasma after smoke vapor exposure will facilitate future behavioral and toxicological experiments in animals, and may prove useful in predicting cotinine levels in human during smoking vaping.

ABSTRACT BOOK

REDUCED RISK NICOTINE PRODUCTS vs CIGARETTE SMOKE: COMPARISON OF THEIR EFFECTS IN THE RESPIRATORY TRACT AND THE ADIPOSE TISSUE OF MICE

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Cigarette smoking constitutes a significant cause of premature death and disease in modern society. Smoking cessation has been proven to be hard for smokers, and long-term abstinence rates remain low, even after the administration of nicotine replacement therapy. Electronic cigarette (e-cigarette) has been recently developed as an alternative product, aiming to reduce conventional tobacco smoking harm. The commercial success of e-cigarette devices is attributed to their ability to a) deliver the major addictive component to smokers, i.e. nicotine, and b) provide a vaping experience that resembles smoking a cigarette.

The aim of this experimental study, using mice, is to investigate the effect of inhaled e-cigarette emissions on lung and adipose tissue and to compare it with exposure to conventional cigarette smoke.

Experimental groups of mice are exposed daily to e-cigarette aerosol, tobacco cigarette smoke or ambient air inside a specially modified inhalation chamber, for up to 8 weeks. A method to accurately determine the levels of nicotine and its main metabolite, cotinine, in animals' plasma using LC/MS-MS has been successfully validated, and serves as a measure of animal exposure levels to the products tested. At the end of the exposure period, mice are euthanized and bronchoalveloar lavage fluid (BALF), lung tissue and adipose tissue are isolated and analyzed to determine a) the leukocyte population profile in BALF by flow cytometry, b) the expression of a number of pro-inflammatory cytokines in lung tissue by ELISA and c) the expression, using Real-time PCR, of marker genes characteristic of brown or white adipocyte phenotype and function in various fat deposits.

Our data will help us determine whether e-cigarette exposure differs from conventional cigarette in terms of eliciting inflammatory responses in the lung and BALF and in modifying adipose tissue profile and thus metabolic function.

ABSTRACT BOOK

NETWORK ANALYSIS FOR THE PRECLINICAL EVALUATION OF CHEMICALS: APPLICATION IN PRIMARY BRONCHIAL EPITHELIAL CELLS AS IN-VITRO MODELS FOR THE EFFECT OF TOBACCO PRODUCTS

Nikos Tsolakos[1], Asier Antoranz[1,2], Chris Fotis[2], Vicky Pliaka[1], Michalis Fragkos[1], Angeliki Minia[1], **Leonidas G. Alexopoulos[1,2]**

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Tobacco products including new products of modified risk (MRTPs) require rigorous evaluation to demonstrate their ability to reduce harmful effects on users. In this context, *in vitro* systems toxicology approaches applied for preclinical evaluation of novel pharmaceutical compounds can offer an ideal platform for assessing biological effects of tobacco products (Talikka et al., Expert Opin Drug Discov. 2017).

Here, we showcase network-based approaches that combine *-omics* data with computational tools to facilitate compound efficacy (Fotis et al., Drug Discov Today, 2018) and biomarker analysis (Antoranz et al., Drug Discov Today, 2017). Joint analysis at the pathway and network level can reveal cellular responses and causative mechanisms in relation to exposure to certain compounds as well as links to disease signatures. We provide examples of the use of such tools in primary bronchial epithelial cells as an *in vitro* model widely used for assessing tobacco-induced effects on lung biology (Rhrissorrakrai et al., Bioinformatics 2015; Poussin et al., Sci Data, 2014).

The development of novel tobacco products can thus greatly benefit from harnessing network-based approaches for risk assessment and identification of biomarkers to inform short and long-term product use.

ABSTRACT BOOK

DETECTION AND QUANTIFICATION OF NICOTINE AND ITS METABOLITES IN SALIVA OF SMOKERS USING RAMAN SPECTROSCOPY

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Detection of nicotine in biological fluids can be the subject of study for many disciplines, such us toxicology. This type of analysis is usually performed with High Performance Liquid Chromatography and Gas Chromatography - Mass Spectrometry. Their advantages include precision, low detection limits, but they are expensive, time-consuming and require expertise.

In this study, Raman Spectroscopy was used as an analytical tool for the detection and quantification of nicotine and its major metabolite, cotinine, in saliva of smokers. Speed and easiness of analysis combined with the possible use of portable systems equipped with optical fibers, are some of the benefits. Samples of saliva from regular-smoking volunteers were collected. Raman spectra were acquired from a saliva droplet, without previous pretreatment, placed on a highly reflective gold coated glass slide following sample dehydration. The characteristic vibration peaks of nicotine at 923 cm^{-1} and cotinine at 750 cm^{-1} were used for their identification in saliva samples. In order to quantify the metabolite cotinine, the standard addition method was used.

The levels of cotinine in smoker's samples varied between 0.2 and 0.4 mg/ml. The concentrations measured were verified employing UV-Vis spectroscopy on the same samples.

ABSTRACT BOOK

AN APPROACH TO NAVIGATE THE U.S. FOOD AND DRUG ADMINISTRATION'S PREMARKET TOBACCO PRODUCT APPLICATION PATHWAY: E-VAPOR PRODUCTS

Willie J. McKinney, William P. Gardner, Maria Gogova, Edward G. Largo, K. Monica Lee, Douglas R. Oliveri, and Donna C. Smith

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Both public health and FDA acknowledge a continuum of risk among tobacco products, cigarettes being the most harmful and lower risk non-combustible tobacco products on the other end of the spectrum. Lower risk products should complement, but not compete, with the proven strategies of harm reduction which include reducing initiation and promoting cessation. Reducing the harm caused by tobacco products is a strategic focus for our companies and an important step in achieving Altria's Mission. We are committed to develop and seek market authorization from FDA for innovative lower-risk products. The current U.S. regulatory environment requires e-vapor product manufacturers to submit Premarket Tobacco Product Applications (PMTAs) by August 2022 to market their products.

We will present a scientific evaluation framework which includes product hazard identification, human exposure assessments and perception and behavior evaluations. This scientific evaluation framework comprehensively addresses regulatory requirements set forth in the PMTA provisions of the U.S. Food, Drug and Cosmetic Act.

ABSTRACT BOOK

ANALYSIS AND DEVELOPMENT OF HEALTH POLICIES USING MODERN INFORMATICS TOOLS: PRODUCTS THAT REDUCE THE HEALTH RISKS OF TOBACCO USE

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Electronic cigarettes have been steadily increasing in popularity among young adults since their introduction to EU markets. E-cigarette companies are targeting many stakeholder groups including regulators, politicians, health bodies, etc.

Our objective was to review the current legislation in Greece, aiming to shape health policies, focusing on e-cigarettes as a means of reducing and ceasing nicotine intake.

We used the computerised version of PolicyMaker for health policy making. It served as a means of collecting and organising important information about the major groups involved with the policy, such as their level and area of activity, their initial position, their influence and their interests.

Our results show that most of the stakeholders express positive opinions about e-cigarettes and support their use as an alternative means of ceasing or reducing smoking. The highly opposed groups do not change their initial positions due to financial reasons. The main current obstacle in Greece is the lack of financial resources. Despite this fact, our policy presents a significant probability of success. With the right strategies, there will be an increase in our policy's supporters and a decrease in the intensity of some stakeholders' opposition.

ABSTRACT BOOK

EVIDENCE-BASED REGULATION

Dr Kgosi LETLAPE

Africa Harm Reduction Alliance (AHRA)

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Thanks to increasingly restrictive laws designating where people can – and mostly can't – light up, smoking has hit a record low in South Africa. Yet, the WHO data corroborates that 1.3 billion smokers will still be puffing away in 2025 globally. So what are we missing to drive these numbers to naught?

The government believes further measures are in order but many healthcare providers are finding it tough to get the committed smoker to stop. Admittedly, smoking is a vice but to a committed smoker it is his lifestyle choice. Many smokers are well aware that quitting is the best option yet they choose to continue smoking and no doubt will continue despite even the more stringent measures being proposed in the draft bill.

What these smokers are not aware of is the emerging, novel products which will still satisfy their need to smoke but have the potential to be less harmful to them, their loved ones and the environment. As a committed partner in improving my patients lives, I want to see the draft bill open up the opportunity to engage with all vested stakeholders. And more importantly see how regulatory measures can be successful by not only restrictive actions but also remaining open and understanding how technology and innovation can help us gain greater momentum in achieving better health for all.

We recognize that the regulators plight to impact non communicable disease prevalence is not an easy task however the medical field hails its successes on new advances, education and awareness. And I say, let us carefully evaluate the role of novel products which show evidence of reduction in harm instead of lumping them together in one basket.

Awareness and education of switching to less harmful alternatives, working with all stakeholders to find the middle ground – in my opinion – and remaining open to emerging science should be the prime focus of the regulators as they open the legislation for amendment.

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So how do we go about finding the middle ground? In my presentation I want to propose we ask to explore a different approach. Should we be considering adopting a precautionary principle recommendation?

Precautionary Principle (PP) looks to prevent possible harm to human health and environment. It has gained support in the international community as a higher-order legal principle that should guide public policy and the formulation of specific laws. But it is also the target of much criticism, with many arguing that the principle is vacuous, inconsistent or based on an excessively conservative attitude towards risk.

Assessment is needed on giving guidance on the nature of precautionary action, and in so doing advised what measures should be:

- proportional to the chosen level of protection;
- non-discriminatory in their applications;
- consistent with similar measures already taken;
- based on an examination of the potential benefits and costs of action or lack of action;
- subject to review, in light of new scientific data that suggest reduced risk;
- capable of assigning responsibility for producing the scientific evidence necessary for a comprehensive risk assessment.

I agree with the author who quoted that the first three points placed the precautionary principle within the broader EU legal framework. The fourth point made it clear that decisions on its application should not be based solely on assessment of the potential hazards. The last two points developed the idea that use of the precautionary principle is contingent on future data (which could either reinforce or contradict the need for protection) and that it is desirable to move toward a full evidence-based risk assessment. So why is such an approach disregarded over the necessity to place the burden of proof on the proponent?

ABSTRACT BOOK

ESTIMATION OF SECOND HAND EXPOSURE LEVELS FROM ENDS AND CONVENTIONAL CIGARETTE USE USING COMPUTATIONAL MODELING

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Most indoor air quality models reported in the literature are well-mixed models. A well-mixed model estimates the room average concentration of constituents from sources. It does not provide information on (1) how far and how fast the emitted chemicals travel in the indoor space, and (2) how the concentration changes as a function of distance from the emission source.

We developed a distributed model (in addition to previously developed well-mixed model), using computational fluid dynamics and thermodynamics principles, which allows for aerosol dispersion in an indoor space and includes evaporation and condensation of constituents in a multi-compound aerosol mixture. The distributed model can estimate the spatial and temporal variations of the concentration of individual constituents present in the emitted aerosol in vapor and particulate phases separately. Results from the model were compared with the published experimental data and were found to be in good agreement. A sensitivity analysis was performed to evaluate the impact of various parameters that affect the air level of the emitted constituents within an indoor space, including rate of emission, the rate of air exchange, etc. Finally the well-mixed model was used to estimate the level of second hand exposure in several confined spaces where e-vapor products (EVPs) or cigarettes were used.

ABSTRACT BOOK

ALDEHYDE EMISSIONS IN RELATION WITH RESISTANCE TEMPERATURE LEVELS AND AGING IN E-CIGARETTES

Nikolaos Vlachos[1], Mohamed A. El Mubarak[2], Charikleia Danika[2], Konstantinos Farsalinos[1], Eugenia Katsoulakou[1], Gregory Sivolapenko[2], and Konstantinos Poulas[1]

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One of the main health concerns about electronic cigarette (EC) usage is the potential for toxic aldehyde emissions, such as formaldehyde, acetaldehyde and acrolein, which are known to be formed from heating mixtures of propylene glycol (PG) and vegetable glycerin (VG), typical carrier solvents for EC liquids (e-liquids), as thermal decomposition products. The levels of the carbonyl emissions are highly dependent on the temperature of the heating coil.

The objective of this study was: a) To explore the carbonyl emissions of a mixture of PG and VG (50:50) in different voltage settings and consequently to higher vaping temperatures, and b) To investigate how the aging of the resistance influences the emission levels of the aldehydes. A Nautilus 2, 0.7 Ω resistance was used and the atomizer was tested at 3.7V, 4.3V and 5V.

Carbonyl emission levels are found to rise in higher voltage settings. Particularly, at 5V the carbonyl levels are extremely high due to the elevated temperature leading to «dry puff» phenomenon. In addition, high voltage settings appeared to be detrimental to the resistance's life time. In particular, at 3.7V and 4.3V a sharp increase in aldehyde emission was observed at approximately 1080 puffs, while at 5 V a similar raise was observed around 80 puffs.

ABSTRACT BOOK

DETECTION AND QUANTITATIVE DETERMINATION OF HEAVY METALS IN ELECTRONIC CIGARETTE REFILL LIQUIDS AND AEROSOLS USING TOTAL REFLECTION X-RAY FLUORESCENCE SPECTROMETRY

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Electronic cigarettes are considered healthier alternatives to conventional cigarettes containing tobacco. They produce vapor through heating of the refill liquids (e-liquids) which consist of propylene glycol, vegetable glycerin, nicotine (in various concentrations), water and flavoring agents. Heavy metals may enter the refill liquid during the production or the aerosol during vaporization, where the liquid is in contact with the metallic resistance of the steaming device at high temperature. Those metals may ultimately be inhaled by the users with potential toxic effects on their health.

In the present study, a methodology was developed for the detection and quantitative analysis of Cadmium (Cd), Lead (Pb), Nickel (Ni), Copper (Cu), Arsenic (As) and Chromium (Cr) in e-liquids and aerosols generated from commercially available atomizers after liquefaction in nitric acid solution. Total Reflection X-Ray Fluorescence Spectroscopy (TXRF) was employed as an alternative technique to ICP-MS or ICP-OES commonly used for this type of analysis. TXRF was chosen due to its advantages, which include short analysis time, promptness, simultaneous multi-element analysis capability and minimum sample preparation, low purchase and operational cost.

The proposed methodology was applied to a large number of commercially available electronic cigarette liquids, their constituents, as well as in aerosols generated from commercially available atomizers after liquefaction in nitric acid solution.

The refill liquids tested were proved to be well below the concentrations defined by regulatory authorities for inhalant medicines. For some of the constituents, nicotine and flavorings, the metals measured surpassed the limits but the dilution to take place will diminish the final quantity and potential risk. On the other hand, in liquefied aerosols from most atomizers, the concentrations determined were within acceptable range with the remarkable exception of vapors generated from atomizers that had undergone extensive use where the levels where remarkably increased.

ABSTRACT BOOK

DEVELOPMENT OF AN ASSAY TO ASSESS GENOTOXICITY BY PARTICULATE MATTER EXTRACT

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The current study describes a method for assessing the oxidative potential of common environmental stressors (ambient air particulate matter), using a plasmid relaxation assay where the extract caused single strand breaks, easily visualized through electrophoresis.

This assay utilizes a tiny amount (11μg) of particulate matter (PM) extract compared to other, cell-based methods (~3000μg). The negative impact of air pollution on human health has been extensively recognized. Among air pollutants, PM holds an eminent role reflected in the broad scientific and regulatory interest. PM toxicity highly depends on its composition (metals and organic compounds), which in turn has been linked to multiple health effects (such as cardiorespiratory diseases and cancer) through multiple toxicity mechanisms; oxidative stress induction is considered one major mechanism among them. In this study the PM levels, the oxidative potential, the cytotoxicity and the genotoxicity of PM in the region of Larissa were examined using the plasmid relaxation assay. Finally, coffee extracts from different varieties, derived from both green and roasted seeds, were examined for their ability to inhibit particulate matter induced DNA damage. These extracts also displayed an inhibitory activity towards xanthine oxidase and catalase, while having no effect against superoxide dismutase.

Overall, the study highlighted the importance of assays for assessing the oxidative potential of widespread environmental stressors (PM) and tobacco particulate matter, as well as the antioxidant capacity of beverages and food items, with the highlight being the development of a plasmid relaxation assay to assess the genotoxicity caused by PM using only a tiny amount.

ABSTRACT BOOK

CHEMICAL COMPOSITION OF *my*blu™ POD-SYSTEM E-CIGARETTE AEROSOLS: A QUANTITATIVE COMPARISON WITH CONVENTIONAL CIGARETTE SMOKE

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Electronic cigarette aerosol is understood to provide reduced exposure compared to conventional cigarette smoke, as it delivers nicotine and flavours without burning tobacco. While recent studies show that e-cigarette aerosol is chemically simple when compared to cigarette smoke, comprehensive analytical assessments of many widely available products are limited.

In this study, the aerosols generated by two commercially available *my*blu™ e-liquids (1.6% nicotine, tobacco flavour; 1.6% nicotine, menthol flavour) in a *my*blu™ pod-system e-cigarette device were analysed and compared to published data for conventional cigarette smoke. The *my*blu™ products were analysed for the principal e-liquid ingredients (nicotine, propylene glycol, glycerol and water) as well as 51 select constituents of public health interest (carbonyls, phenolics, volatile organic compounds [VOCs], metals, tobacco-specific nitrosamines [TSNAs], polyaromatic amines [PAAs], and polycyclic aromatic hydrocarbons [PAHs]). The select constituents include those on the FDA Harmful or Potentially Harmful Constituents (HPHCs) list of chemicals in cigarette smoke it considers cause or could cause harm to smokers.

The e-cigarettes were puffed in two separate 50-puff blocks using the CORESTA Recommended Method CRM81 (puffing regime: 55mL/3sec/30sec; square wave). Five replicates were measured for each e-liquid type. All measurements were conducted by an independent laboratory.

The data shows the substantial chemical differences between commercially available e-cigarettes and conventional cigarettes: the aerosol from the *my*blu™ pod-system e-cigarettes is less complex than cigarette smoke and contains significantly lower levels of toxicants and carcinogens. Of the 51 toxicants analysed, eight were observed at quantifiable levels, including formaldehyde, acetaldehyde and acrolein (>99% reduction vs. conventional cigarette); manganese and selenium (average 82% reduction vs. conventional cigarette); and NNN, NAT

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and NNK (>99% reduction vs. conventional cigarette). Under the test conditions, the total yield range was 0.96-0.97 µg/puff of toxicants tested for the *my*blu™ flavours, which is 99% less than the approximate 381 µg/puff quantified and published for conventional cigarette smoke.

These data demonstrate that high quality e-cigarettes and e-liquids offer the potential for substantially reduced exposure to cigarette carcinogens and toxicants in smokers who use such products as alternatives to cigarettes.

ABSTRACT BOOK

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODOLOGY FOR THE QUANTIFICATION OF ALDEHYDES IN E-CIGARETTE AEROSOLS USING UHPLC-UV

Mohamed A. El Mubarak[1], **Charikleia Danika[1],** Nikolaos S. Vlachos[2], Konstantinos Farsalinos[2], Konstantinos Poulas[2], and Gregory B. Sivolapenko[1]

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An electronic cigarette (e-cigarette) device is comprised by a battery, as a power unit, and an atomizer that includes the heating coil, which produces the steam. Most commonly, e-liquids contain water, nicotine, vegetable glycerin (VG), propylene glycerol (PG) and a mix of flavoring additives, in variable concentrations, in order to achieve the desired aroma while vaping. In recent years, e-cigarettes have emerged as a prevalent substitute to conventional cigarettes. Vaping seems to be safer, although, studies indicate that toxicants are produced due to the thermal decomposition of the e-liquids.

The objective of this study is to develop a sensitive, selective and reproducible UHPLC-UV method for the determination of aldeydes in base liqiuids (PG-VG) and some flavoring compounds. Aldehydes are produced in e-cigarette aerosols, due to the thermal decomposition of vegetable glycerin, propylene glycerol and flavorings in the atomizer. These aldehydes were collected with derivatization into 2,4-dinitrophenylhydrazine using impinger trapping. A UHPLC methodology for simultaneous quantitative of aldehydes in base liquids and flavorings was developed. Chromatographic separation was carried out on a Jupiter Proteo 90A column, with the mobile phase consisting of 0.1% formic acid in water and acetonitrile, at a flow rate of 0.4 mL/min.

Linearity was demonstrated over the range of concentrations 0.025-10 μg/mL. The lower limit of detection and quantification were 0.008 and 0.025 μg/mL, respectively. As a result, it has been shown that vegetable glycerin produces the highest percentage of aldehydes after thermal decomposition compared to propylene glycerol. Furthermore, after the addition of flavors in base liquids it was found that some flavoring compounds can exponentially increase the generation of aldehydes.

A comparison of aldehyde concentrations in flavored compounds shows that, chocolate mousse product the highest aldehyde concentration compared to strawberry and prince.

ABSTRACT BOOK

THE IMPACT OF E-CIGARETTE FLAVORING AGENTS IN ALDEHYDE EMISSIONS

Charikleia Danika[2], Nikolaos Vlachos[1], Mohamed A. El Mubarak[2], Zoi Zagoriti[1], Konstantinos Farsalinos[1], George Lagoumintzis[1], Eugenia Katsoulakou[1], Gregory Sivolapenko[2], and Konstantinos Poulas[1]

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Toxic aldehyde emissions, such as formaldehyde, acetaldehyde and acrolein, formed from heating mixtures of propylene glycol (PG) and vegetable glycerin (VG), as well as flavoring compounds, typical carrier solvents for EC liquids (e-liquids), is one of the main health concerns about electronic cigarette (EC) usage, since these aldehydes are potential irritants, toxicants and/or carcinogens. In particular, recent studies have proved that e-liquid flavors are the major factor for aldehyde emissions. Dramatic differences are also observed among different flavors.

The aim of this study was to explore the effect of flavoring compounds in acetaldehyde, acrolein, crotonaldehyde and formaldehyde emissions. The e-liquids used can be categorized in three groups: Fruit, tobacco and sweet flavors. A Nautilus 2, 0.7 Ω resistance was used and the atomizer was tested at 3.7V.

Sweet flavors were found to exhibit the highest levels of aldehyde emission than the other two types. The carbonyl emission between fruit and tobacco flavors seem to be similar. It is worth mentioned, that even among flavors of the same category, intense variations were observed.

ABSTRACT BOOK

TOBACCO-SPECIFIC NITROSAMINES

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Smoking is the leading cause of human cancer and other related preventable diseases. Cigarette smoke is a complex mixture of chemicals, such as tobacco-specific nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs), which are compounds that have tumorigenic and mutagenic activities. Most TSNA are formed in tobacco during the post-harvest period, while a number are produced when a cigarette is burned. Considerable evidence supports the role of TSNA important causative factors for cancers of the lung, pancreas, esophagus, and oral cavity in people who use tobacco products.

This study refers to the most known TSNAs such as nitrosoanabasine (NAB), nitrosoanatabine (NAT), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and nitrosonornicotine (NNN). Other nitrosamines include 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) and 4-(methylnitrosamino)-4-3-pyridyl) butyric acid (iso-NNAC). Of the known TSNA, NNK and NNN are considered the most carcinogenic.

New tobacco products (e.g., e-cigarettes) designed to attract consumers who are concerned about the health effects of tobacco have been appearing on the market. Several studies have reported that certain TSNA have been detected in the replacement liquids and vapour of e-cigarettes, but the levels are generally considerably lower than in tobacco cigarettes. In conclusion, the e-cigarettes are a compromise solution for reducing the exposure to tobacco smoke toxicants.

ABSTRACT BOOK

CARBONYL EMISSIONS FROM A HEATED TOBACCO PRODUCT (IQOS) COMPARED WITH AN E-CIGARETTE AND A TOBACCO CIGARETTE

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Heated tobacco products have been recently marketed as harm reduction products, with limited independent research on their emissions. The aim of this independently-funded study was to compare carbonyl emissions from a heated tobacco product widely available in Europe (IQOS) and compare them with a commercial tobacco cigarette and a new generation e-cigarette.

Aerosol and smoke was collected using an automated machine in 2 impingers connected in series that contained DNPH. The e-cigarette was tested at 2 power settings (10 W and 14 W). Health Canada Intense (HCI) and two more intense puffing regimes were tested. Carbonyl emissions were measured using HPLC.

IQOS regular and menthol IQOS emitted 6.4 and 5.0 μg/stick formaldehyde, 144.1 and 176.7 μg/stick acetaldehyde, 10.8 and 10.4 μg/stick acrolein, 12.8 and 11.0 μg/stick propionaldehyde and 2.0 and 1.9 μg/stick crotonaldehyde. IQOS products emitted approximately 90% less formaldehyde, 85% less acetaldehyde, 90% less acrolein, 90% less propionaldehyde and 95% less crotonaldehyde compared to the tobacco cigarette. The e-cigarette emitted approximately 98% less formaldehyde, 99.9% less acetaldehyde and 99.5% less acrolein compared to the tobacco cigarette. Propionaldehyde and crotonaldehyde were not detected in the e-cigarette aerosol. At more intense puffing regimes, formaldehyde emissions were increased in IQOS products but were still approximately 70% lower compared to the tobacco cigarette; all other carbonyls were emitted at levels similar to the HCI regime. The levels of carbonyls measured from IQOS herein were similar to those reported by the manufacturer in the literature.

IQOS emits substantially lower levels of carbonyls compared to a commercial tobacco cigarette but higher levels compared to an e-cigarette. The study identifies a risk continuum between different harm reduction products.

ABSTRACT BOOK

MEASUREMENT AND DISTRIBUTION OF AEROSOL PARTICLE SIZE OF COMMERCIAL LIQUIDS IN E-CIGARETTE EMISSIONS: A COMPARISON WITH CONVENTIONAL CIGARETTE PRODUCTS

Spyros Lampos[1], George Lagoumintzis[1], Evaggelia Kostenidou[2], Eugenia Katsoulakou[1], Zoi Zagoriti[1], Aristeidis Ntoukas[1], Konstantinos Dalamarinis[1], Panagiotis Savranakis[1], and Konstantinos Poulas[1]

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Cigarette smoke is a complex mixture of chemicals, many of which are generated during the processes of tobacco combustion. Because of the absence of tobacco leaf combustion, electronic cigarettes (ECs) are expected to produce less harmful compounds. However, concern exists regarding the potential passive exposure to the aerosol exhaled by EC users.

Herein, the aerosol particles (~2.5 μm in diameter) produced by different commercial EC liquids in real time were measured and compared to those of conventional cigarettes. Two individual experiments were conducted. In the first, the mass and the amount of aerosol particles from 6 different EC liquids that were produced through vaping process were measured in a closed room of 35 m^3 volume. The second experiment was held in a 40 lt experimental chamber and divided into 2 phases. During the first phase, the particles produced through vaping process were defined. In the second phase, after overheating the atomizer, the volatile organic compounds (VOCs) produced were measured.

Our results depict that aerosol from EC liquids has a different emission particulate profile than that of commercial cigar and hand rolling cigar. In detail, ECs instantly produce higher mass and number aerosol concentrations than commercial and hand rolling cigars do. EC aerosols have very small life time ~10-20 sec. They volatilize instantly as they probably consist almost only of PG and VG. Cigars produce quite high aerosol mass and number concentration with much longer life time (dissipation time ~1.5 h in a 35 m3 room).

The analysis of the aerosols showed that the only harmful substance in EC is CO, however its quantities are far lower than the traditional cigarette and are considered safe. Finally, when the atomizer was overheated, the number of VOCs produced, exceed the minimal risk level defined the Environmental Protection Agency.

ABSTRACT BOOK

DEVELOPMENT OF A RAMAN SPECTROSCOPY METHOD FOR THE ON-LINE ANALYSIS OF NICOTINE IN ELECTRONIC CIGARETTE REFILL LIQUIDS

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Electronic cigarettes allow users to inhale an aerosol containing nicotine and have gained great acceptance during recent years as a healthier alternative to cigarettes containing tobacco. These battery operated devices contain a heating element to cause vaporization of specially produced refill solutions. An electronic cigarette refill liquid is consisted of humectants (usually propylene glycol or vegetable glycerin), flavoring agents and nicotine at concentrations ranging from 0 mg/mL to 24-36 mg/mL.

Quantification of nicotine is mainly performed by the techniques of High Performance Liquid Chromatography (HPLC) and Gas Chromatography conjugated with Mass Spectrometry (GC/MS). In the current study, an alternative method based on Raman spectroscopy was developed for the quantitative determination of nicotine in solutions used in electronic cigarette devices. This particular technique is widely used due to its ease of operation and rapidness. A portable Raman spectrometer equipped with an optical fiber probe was used for spectra acquisition, enabling the on line analysis, i.e. during the production process, and data collection through sealed containers without sampling.

A large number of electronic cigarette refill liquids commercially available on the free market were analyzed using the method of standard solutions. The calibration curve showed good linearity and relative errors from the values indicated in the package were found to be up to 10%. The proposed methodology is characterized by good repeatability and the lower limit of quantification is 1.22 mg/mL. The results were compared with those obtained from GC.

ABSTRACT BOOK

DETECTION OF HEAVY METALS IN ELECTRONIC CIGARETTE AEROSOLS USING TOTAL REFLECTION X-RAY FLUORESCENCE SPECTROMETRY

Eleni Kamilari[1], Konstantinos Farsalinos[2,3], Athanasios Niarchos[2], Evangelia Konstantinou[2], **Konstantinos Poulas[2]**, Christos G. Kontoyannis[1,4], and Malvina G. Orkoula[1]

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Heavy metals may enter the solution that is vaporized in electronic cigarettes devices (electronic cigarette refill liquid) during the production process or the aerosol during vaporization, where the liquid is in contact with the metallic resistance of the steaming device at high temperature. Those metals may ultimately be inhaled by the users with potential toxic effects on their health.

In the present study, a methodology, based on Total Reflection X-Ray Fluorescence Spectroscopy (TXRF), was developed for the detection and quantitative analysis of heavy metals in aerosols generated from commercially available atomizers after liquefaction in nitric acid solution.

Results showed that nickel, lead, copper and chromium were detected in electronic cigarette refill liquids and their concentrations were within the range of 0.001 to 0.055 ppm. Cadmium was identified in a sample of electronic cigarette liquid (0.655 ppm), as well as in nicotine and in some kinds of flavoring agents. Nickel, lead, copper and chromium were determined in liquefied aerosols from most atomizers in concentrations within the range of 0.001 to 0.009 ppm, whereas their levels were higher (0.013-0.106 ppm) in liquefied vapors generated from atomizers that had undergone extensive use.

ABSTRACT BOOK

ALTERNATIVE TOBACCO PRODUCTS: TOXICOLOGY AND HEALTH ISSUES

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The last few years many smokers have replaced smoking with new ways of delivering nicotine without combustion. New technologies and devices, such as Electronic Cigarettes and Heat-not-Burn products are available and are collectively called Tobacco Harm Reduction (THR) products, giving a potentially safer alternative to smoking. However, this must be evaluated.

Occasioned by the special issue "Alternative tobacco products: Toxicology and Health Issues", published in *Food and Chemical Toxicology* journal we are giving a synopsis of the results, as presented in the 13 articles of this special issue.

ABSTRACT BOOK

APPROACHES TO INVESTIGATE DYNAMICS AND DELIVERY OF MULTISPECIES EVOLVING LIQUID AEROSOL MIXTURES ALONG THE HUMAN RESPIRATORY TRACT

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Despite continuous progress in development of technologies allowing us to measure, characterize, and assess aerosol flows, many aerosol dosimetry phenomena in particular pertaining to the dynamics and evolution of liquid aerosols entering the human respiratory tract continue to challenge the scientific community. The complexity of this research domain can be attribute to factors ranging from the lack of standardized experimental approaches for *in vitro* aerosol exposure testing under human relevant exposure conditions, difficulties in developing radiolabeled aerosols, as well as the lack of validated computational models able to predict both the chemistry and physics of aerosols. Multidisciplinary efforts including expertise from aerosol science, physics, and experimental inhalation toxicology provide opportunities to jointly establish protocols to investigate the dynamics, delivery and deposition of aerosols entering the human airways and ultimately predicting the effective delivered doses. The approaches and methodologies applied are equally pertinent in toxicity assessment of aerosols as well as for targeted drug delivery strategies.

We present our multidisciplinary approaches employed to understand the dosimetry of multispecies evolving liquid aerosol mixtures in the context of *in vivo, in vitro* and *in silico* aerosol research. In particular, we concentrate on the following three aspects: 1) Development of a computational fluid dynamic model (AeroSolved) capable to simulate formation, transport, evolution and deposition of aerosols. 2) Application of simulations for *in vitro* and *in vivo* exposure studies helping in characterization of used exposure systems and building predictive analytical models for the aerosol deposition/dosimetry. 3) Development of 3D printed casts for experimental research of aerosol dynamics in human lungs. By simultaneous and systematic use of the developed methods and tools, we hope to gain the much required insight into aerosol dosimetry of multispecies evolving liquid aerosol mixtures.

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