

# A FRAMEWORK FOR THE ASSESSMENT OF REDUCED RISK TOBACCO AND NICOTINE PRODUCTS

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## Abstract

Cigarette smoking is a cause of many human diseases including cardiovascular disease, lung disease and cancer [US Department of Health and Human Services 2014]. The use of novel tobacco and nicotine products with reduced yields of toxicants compared to cigarettes, such as tobacco-heating products, low toxicant oral smokeless products (*eg*, snus) and electronic cigarettes, hold great potential for reducing the harms associated with tobacco use. Currently, however, this harm reduction potential has yet to be scientifically substantiated and the US Food and Drug Administration (FDA), is the only national regulator to have provided a draft framework with which to assess novel tobacco and nicotine products for their harm reduction potential via their Modified Risk Tobacco Product directive [FDA 2012a]. In this paper we describe a framework for the assessment of such products that includes four key assessment phases: stewardship science, exposure reduction, individual risk reduction and population risk reduction. This integrated approach proposes the use of pre-clinical, clinical and population studies to assess the risk reduction potential of new products at the individual and population level.

## 1.0. Introduction

Tobacco products are used on a global scale, with current estimates of 1.4 billion adult cigarette smokers worldwide, [MacKay 2006]. Numerous epidemiological studies have shown that cigarette smoking causes a variety of smoking related diseases such as cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD) and cancer [US Department of Health & Human Services 2014]. Tobacco harm reduction, which was defined by the US Institute of Medicine (IOM) in 2001 as “decreasing total morbidity and mortality, without completely eliminating tobacco and nicotine use” (Stratton 2001) is being considered by some regulators. For example, the FDA through their approach for determining a Modified Risk Tobacco Product (MRTP), either through demonstration of reduced toxicant exposure or reduction in health risks [FDA 2012a].

Product development is currently focussing on novel reduced-risk products, including tobacco heating products (THPs), snus and electronic cigarettes (e-cigarettes). In many countries, including the USA and also European countries,

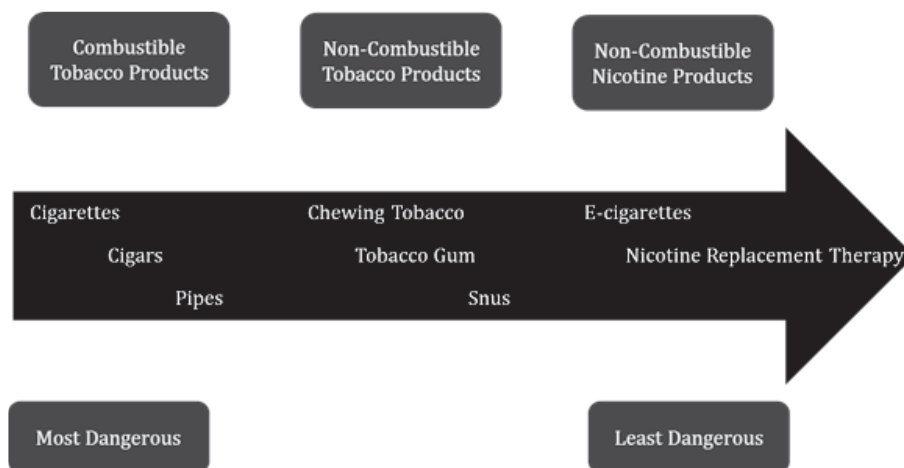
the ability to market such products may be subject to regulatory approvals. These would be obtained by submitting details of a new product's design, performance and impact on users and non-users. Regulators outside the US are considering the need for substantial data packages of pre-clinical, clinical and population studies to be provided for the assessment of novel products. In the USA, these studies form part of an MRTP application [FDA, 2012a], and in Europe they may become part of the requirements with the updated Tobacco Products Directive [Tobacco Products Directive 2014].

Many tobacco products are currently used by consumers, from factory-made cigarettes through shisha to snus, have been in use worldwide for longer than 100 years. Epidemiological evidence, particularly from Sweden, suggests that snus use is substantially less hazardous than cigarette smoking because it is not associated with increased risks of lung cancer, oral cancer and COPD [IARC 2007] and there is active current debate on the role snus could play in tobacco harm reduction. In the last 20 years, products that heat rather than combust tobacco have been marketed in a variety of formats from cigarette shaped products that are lit and used in a similar manner to cigarettes but do not burn down to the more recently developed electrically heated and gas powered systems.

The original concept of an electronic-cigarette was conceived in 1965 by Herbert A. Gilbert [Gilbert 1965], and were more recently commercialised by the Chinese pharmacist, Hon Lik in the mid-2000s. E-cigarettes do not contain tobacco and heat liquid containing nicotine to deliver a plume of aerosol to the consumer. British American Tobacco marketed their first e-cigarette under the brand name Vype in 2013 and today the majority of major tobacco companies market e-cigarettes. These products have evolved rapidly, resulting in a variety of products from single-piece cigarette-like products to modular devices with interchangeable parts, with myriad flavours and unflavoured formulations which can contain nicotine or are nicotine free.

There are currently significant research efforts to determine the risk reduction potential of these novel products and the concept of the risk continuum for tobacco and nicotine products was conceived in 2012 [McNeill 2012]. This continuum included the various product categories and is illustrated in Figure 1.

Figure 1. Concept of nicotine harm continuum



Building on this concept and including tobacco-heating products, we proposed the risk continuum as described in Figure 2 in our sustainability reporting in 2014 [BAT 2014].

Figure 2. Proposed Tobacco and Nicotine Product Risk Continuum

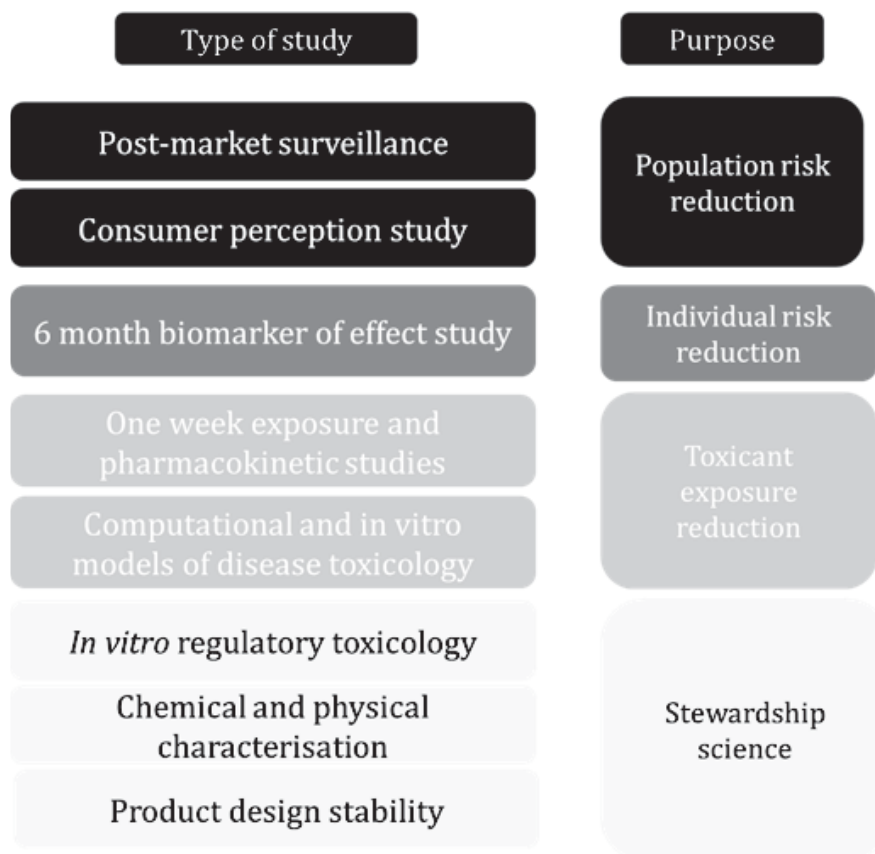


There is a need for a deeper understanding of the science to support the evaluation and to place products across the risk continuum. A harmonised approach to defining a scientific framework with regulatory, public health and industry scientists could create evidence based regulation for these new products and enable the substantiation of health related claims.

In addition to the proposed regulatory assessment framework (*ie*, the US FDA's framework for MRTPs), a recent publication from the Tobacco Product Assessment Consortium (TobPRAC ) presented a four-stage model inclusive of pre-market evaluation; pre-claims evaluation; post-market activities; and monitoring and re-evaluation [Berman 2015]. This framework highlighted key tests and reference products that would be required to demonstrate reduction in risk and product stability by chemical, toxicological and human studies at the individual and population levels.

In this paper we describe a four-phase framework for the scientific evaluation of products across the risk continuum, with a focus on THPs and e-cigarettes. The phases build on each other and propose the integration of pre-clinical, clinical and population studies to assess the risk reduction potential of novel tobacco heating and nicotine products at the individual and population levels. A pre-clinical suite of data is proposed to include product stability, chemical characterisation and as British American Tobacco has publically stated that it does not conduct research on animals unless stipulated by legal or regulatory requirements or public health expectations, particularly in support of new products across the risk spectrum, the toxicological approach is focussed on *in vitro* assays [BAT 2015]. Clinical data would encompass measuring both Biomarkers of Exposure (BoE) and Biological Effect (BoBE) for extended periods of up to six months. To assess population risk reduction, studies proposed include consumer usage and perception in addition to dynamic population modelling in post- market surveillance (Figure 3).

Figure 3. Four phase assessment framework to assess the risk reduction potential of products across the risk continuum



Furthermore, a weight of evidence approach is recommended as the novel tobacco heating and nicotine products would have to demonstrate the potential for risk reduction in each phase versus a comparator product. Importantly, this proposed framework is comprised of a range of pre-clinical, clinical and population studies that would afford an opportunity to review the datasets in totality and not just as independent subsets.

## 2.0 Approach to demonstrating the risk reduction potential of products across the risk continuum

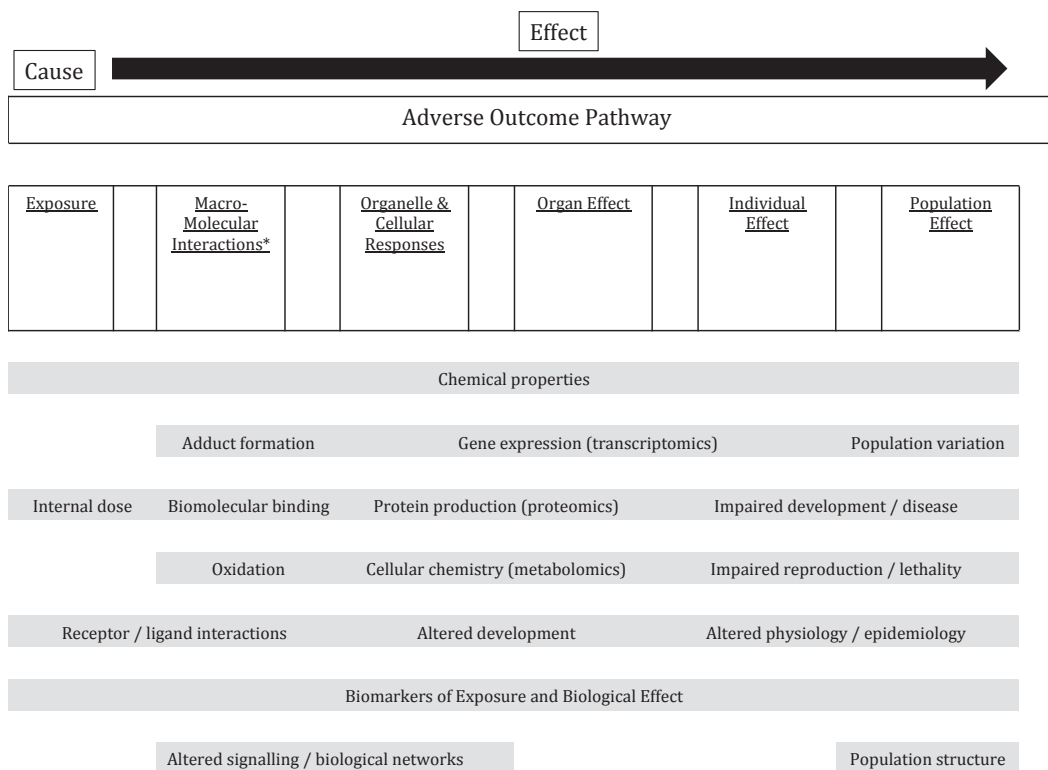
The risk reduction potential of novel tobacco heating and nicotine products versus a comparator product will depend on two factors; (i) the reduction of toxicity, exposure etc. of the product and (ii) the number of smokers who switch, which will depend on the acceptability of the new product in terms of sensory, ritual and use-behaviour. A simple bespoke harm reduction equation was proposed by Bates [Bates 2013] as shown in Figure 4.

Figure 4. Harm reduction equation as proposed by Bates


$$\text{Harm reduction} = \text{Reduced risk} \times \text{Number who switch}$$

In addition to a pre-clinical assessment, data from *in vitro* assays from relevant human tissues (cardiovascular and respiratory) and biomarker studies are required to assess risk reduction. A methodology is required to consolidate all relevant datasets into an organised and mechanistically meaningful information source, to understand adverse effects and to enable assessment of risk reduction potential. The Adverse Outcome Pathway, AOP framework, as defined by Ankley *et al.* [Ankley 2010] is one approach toward providing such an assessment. The principles of this approach were based upon a report from the US National Research Council entitled “Toxicity Testing in the 21st Century” [Andersen 2007]. Under the framework, a “cause and effect” relationship can be established by identifying a molecular initiating event(s), which is linked to an adverse outcome by so-called “key events”, *ie.* measureable changes in biology, which lie along the pathway to the adverse outcome. Such events can be at the molecular, cellular, tissue and whole organ levels and this is illustrated in Figure 5.

Figure 5. Integrated testing approach incorporating pre-clinical, clinical and population studies into Adverse Outcome Pathway methodology



By populating the framework with the data types shown in Figure 5, one can begin to better understand the sequence of events that occurs upon exposure to a given product, the mechanisms responsible for any biological changes, and ultimately, it may be possible to assign quantitative weighting to each key event to help inform risk assessment. The four phase assessment framework to assess the risk reduction potential of reduced risk tobacco and nicotine products will now be explained in further detail.

### 3.0 Phase 1: Stewardship Science

#### 3.1 Product stability

New products undergo a thorough set of tests to determine their stability over time. The products are aged in stability cabinets which maintain a set temperature and humidity, prior to analytical testing. In general the ageing occurs in real time, although elevated temperatures and humidities can be used to accelerate the ageing process in order to provide rapid turnaround of such tests. The analytical tests performed will depend on the nature of the product being tested, but in general such tests can include chemical stability, leakage or loss of ingredients, ingress/absorption of water, leaching of device material into the formulation/tobacco, microbial growth, device performance, cosmetic effects and integrity of packaging. Such tests can be performed at various time-points during the ageing

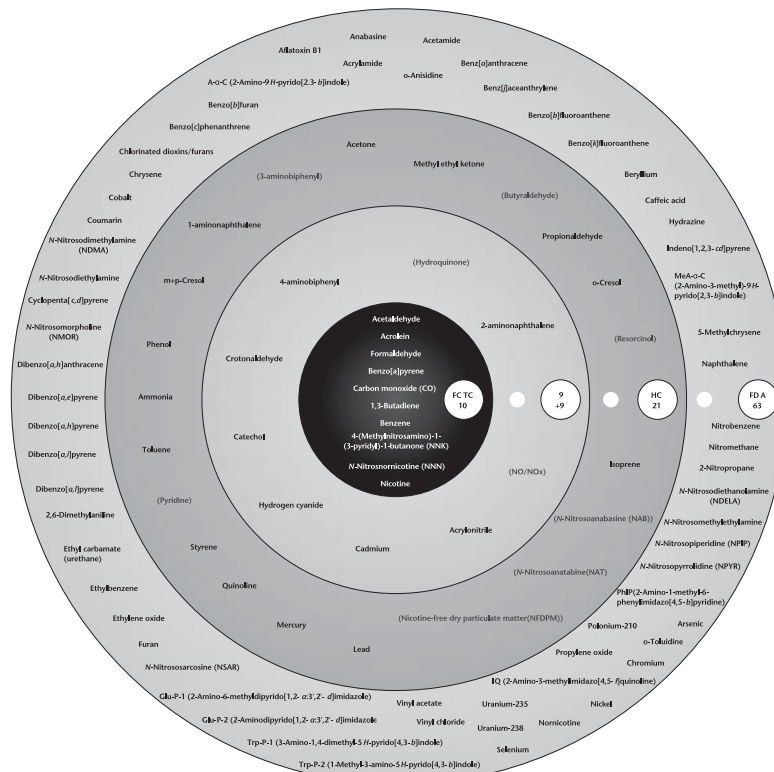
period. The results of this testing will allow the defining a suitable shelf life, and thus ensure that products reach consumers in a good condition.

### 3.2 Chemistry

Tobacco smoke is a complex mixture of gases and volatile, semi-volatile and involatile compounds that have been extensively characterised, *eg.* Rodgman and Perfetti have catalogued the literature and identified over 6000 components of tobacco and tobacco smoke [Rodgman 2013]. Around 150 of these components are known to be toxic to humans and are called “tobacco smoke toxicants” [Fowles 2003]. Initial studies have demonstrated that THPs [Zenzen 2012] and e-cigarettes [Cheng 2014], generate reduced levels of toxicants compared to cigarettes due to the absence of pyrolysis and combustion conditions in both product types and the additional absence of tobacco in e-cigarettes.

The World Health Organization has detailed the way toxicants should be ranked [Burns 2008] and has identified 18 toxicants they prioritise as being of particular concern, including nine for mandatory lowering [WHO Study Group on Tobacco Product Regulation, 2008]. Additionally, the FDA has produced a list of harmful and potentially harmful constituents (HPHCs) in tobacco products and cigarette smoke [FDA, 2012b], as illustrated in Figure 6 below.

Figure 6. Harmful and potentially harmful constituents of products across the risk spectrum



For the demonstration of risk reduction potential it is proposed that the full list of HPHCs should be measured to ensure coverage of the full range of chemical classes, unless it is shown that certain HPHCs cannot be formed in a particular category of product.

### 3.3 *In silico* assessments

As a bridge between stewardship science and exposure reduction, *in silico* assessments are used to assess the potential health risks of individual toxicants. We have developed a quantitative risk assessment method that is scientifically, evidence and biologically based and reflects the range of yields and human use-behaviours in relation to exposure. We use a combination of computer (*in silico*) modelling approaches and *in vitro* experimental data to build models as physiologically relevant as possible to smoking-related diseases.

Margin of exposure (MOE) calculations are used to set concern levels for individual toxicants. This process has been developed for tobacco smoke toxicants on the basis of guidelines developed by the European Food Safety Authority MOE model [EFSA, 2006]. Lower MOE values indicate greater concern. Where possible, multiple dose–response data sets are used to generate an MOE range for a single toxicant that indicates priorities for risk management actions.

Mode of action (MOA) describes how a toxicant affects the body at the tissue or cellular level. MOA reviews are conducted by systematically evaluating data available on a specific response (carcinogenic or not) to a toxicant [International Program on Chemical Safety, IPCS 2008] to establish a biologically plausible sequence of key events, which form the basis of the AOP. The results, supported by robust experimental observations and mechanistic data, help us to inform the quantitative assessment of risk to humans and to identify areas for future research. One of the most technically challenging tasks in assessing the biological effects of exposure to tobacco smoke is to predict the target-tissue concentrations of toxicants. To address this we have developed physiologically-based pharmacokinetic (PBPK) models and recently published an assessment of 1,3-butadiene [Campbell 2015].

Individual toxicants prioritised *in silico* are then assessed *in vitro* using end-points that best represent the underlying MOA for each (genotoxicity and mutagenicity). The concentration of toxicants required to cause biological responses *in vitro* may be contextualised against the predicted target-tissue levels in smokers generated with PBPK modelling.

To improve risk assessment, we are investigating the utility of MOE calculations for a small-scale mixture of three aldehydes and have also conducted MOA reviews on six aldehydes present in smoke with the aim of grouping similar acting



toxicants. This would allow future cumulative risk assessments to be carried out on groups of compounds [Cunningham 2012].

### 3.4 *In vitro* toxicology

A battery of *in vitro* assays has been proposed for toxicology testing (Table 1). This comprises a series of tests that have been used by the tobacco industry for a number of years to assess novel tobacco products and the methodologies have been consolidated across the different companies through the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) association (CORESTA 2015).

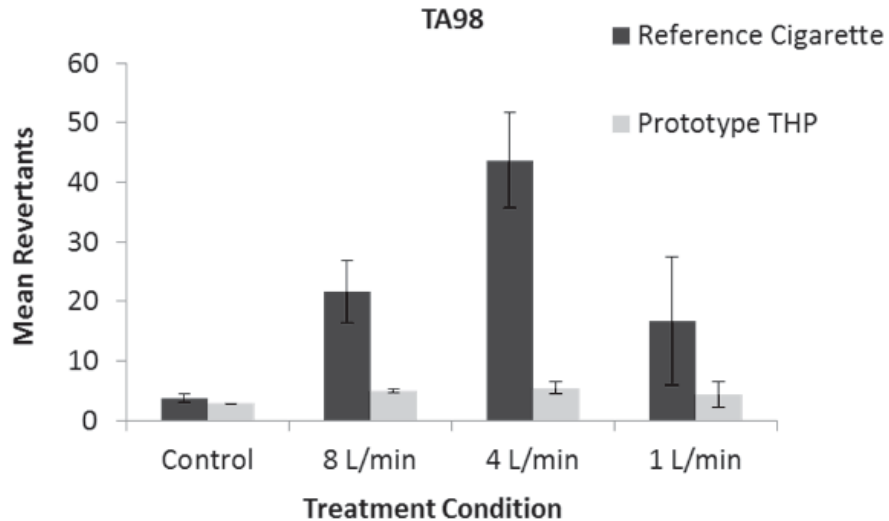
Table 1. Summary of standard *in vitro* toxicological tests proposed assessing novel tobacco heating and nicotine products

Test	End Point	Guideline Reference	Measurement
Ames	Mutagenicity	OECD 471	Demonstrates the extent to which the test article can induce mutation in bacterial cells.
Micronucleus	Genetic damage:- anugenic or clastogenic events	OECD 487	Detects structural changes (aberrations), measured by micronuclei.
Mouse Lymphoma	Mutagenicity and clastogenic events	OECD 476	The mouse lymphoma assay utilises L5178Y cells and exploits the enzyme thymidine kinase to detect gene mutations and chromosome aberrations.
Neutral Red Uptake	Cytotoxicity	ICCVAM publication No. 07-4519	A cell viability assay, based on the ability of cells to incorporate the supravital dye, Neutral Red.

In routine *in vitro* evaluation of combustible tobacco products to date, the particulate fraction (Particulate Matter, PM), from mainstream cigarette smoke has been used as the test article. For the assessment of novel tobacco heating and nicotine products moving forward, it is proposed to use PM in addition to both direct exposure of whole aerosol and exposure to aqueous extracts containing trapped aerosols as they contain both particulate and gas phase constituents. Additionally, the *in vitro* end-points will need to be modified as appropriate for each exposure system.

In a modified AMES assay, bacteria at the air-agar interface were exposed to whole aerosols generated from a reference cigarette (Kentucky Reference cigarette, 3R4F) and a prototype THP, for assessment of mutagenic activity (Figure 7), using the Vitrocell VC 10 smoking robot [Thorne 2015].

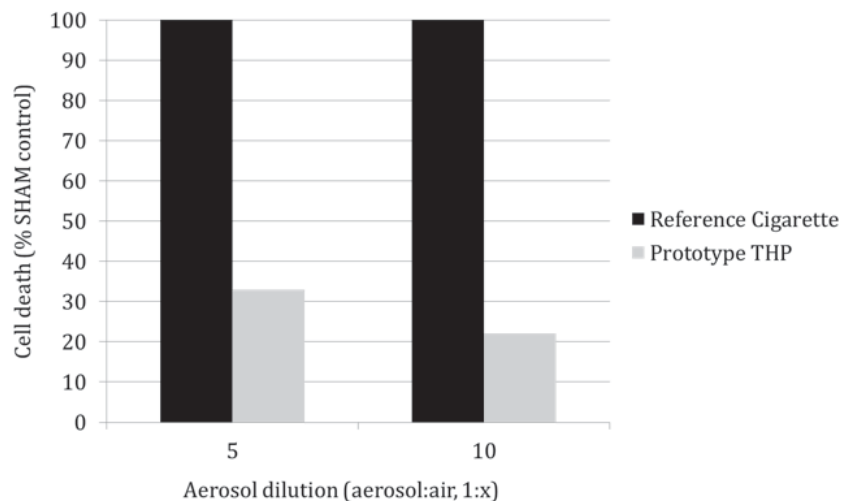
Figure 7. Genotoxicity response for a reference cigarette and a prototype THP using AMES



These AMES results suggest that matched for exposure conditions, cigarette smoke induces a genotoxic response that is not observed with an aerosol from the prototype THP.

In addition to assessing products for mutagenicity, we propose to test for cytotoxic responses using a Neutral Red assay as illustrated in Figure 8.

Figure 8. Cytotoxicity response to a test for a reference cigarette, and a prototype THP



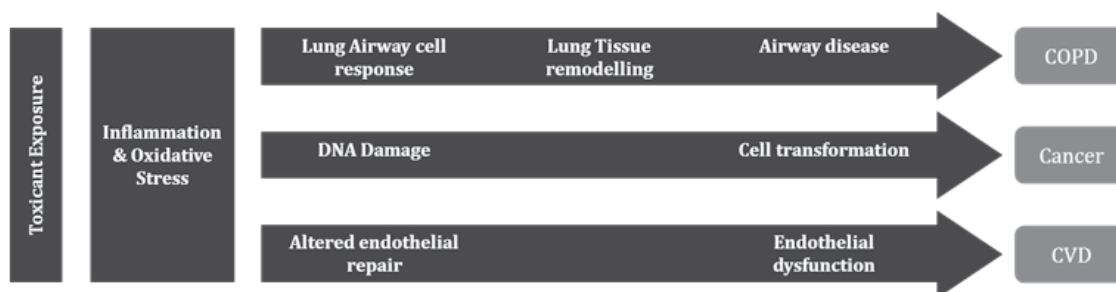
These data indicate that at matched doses, smoke from the reference cigarette induced complete cell death, whereas a substantially reduced response was observed with the aerosol from the prototype THP. The absence or substantial reduction in mutagenicity and cytotoxicity from products across the risk spectrum versus a reference cigarette would be a significant first step in demonstrating a risk reduction potential.

#### 4.0 Phase 2: Exposure Reduction

Using a weight of evidence approach, a successful outcome from Phase 1 would be both measurably reduced chemical toxicant yields and reduced responses from *in vitro* toxicological tests compared to a comparator product. The second phase would assess the novel tobacco heating and nicotine products in more disease-relevant *in vitro* assays and to understand whether machine-based chemistry reductions are likely to translate into reduced disease relevant biological effects observed in humans. Classic toxicological endpoints such as histological, DNA damage, oxidative stress, and cytotoxic markers are valuable tools to evaluate tissue injuries and we routinely quantify those *in vitro* using the COMET assay [Dalrymple 2015],  $\gamma$ -H2AX phosphorylation,[Garcia-Canton 2014] glutathione depletion, free radical formation, and LDH release [Neilson 2015 & Ordonex 2014].

The principles of an AOP approach have been adapted to map the cascade of key events leading to the development of smoking-related diseases (Figure 9) and to support future research developments.

Figure 9: Adverse Outcome Pathway to map key event to smoking related diseases

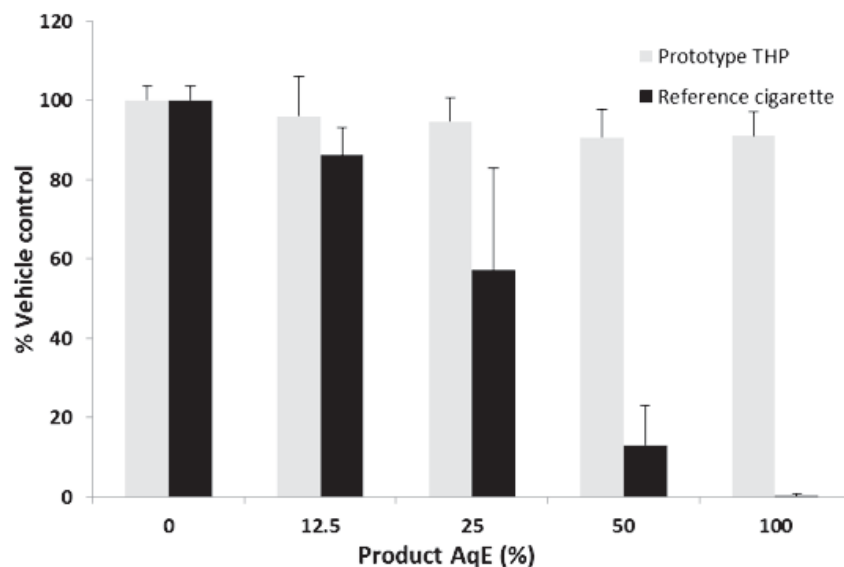


#### 4.1 Inflammation and Oxidative Stress

Toxicant exposure is measured firstly in terms of machine based yields and then in humans via biomarkers of exposure. Inflammation and oxidative stress are important events that often follow exposure to toxicants and are critical for driving processes for the development of COPD, cardiovascular diseases and lung cancer. Inflammation and oxidative stress is assessed by relevant *in vitro* assays, including anti-oxidant depletion, reactive oxidative species (ROS) generation, activation of antioxidant response and inflammatory cytokine secretion. Previously,

experiments were performed examining glutathione levels in lung epithelial cells which were exposed to aqueous extracts of smoke from a conventional and a Reduced Toxicant Prototype (RTP) cigarette [Proctor 2014]. This test was adapted to assess products across the risk continuum via exposure to aqueous extracts of cigarette smoke and aerosol from a prototype THP. The oxidation to glutathione disulphide (GSSG) was measured and the ratio of GSH:GSSG for a reference cigarette and a prototype THP are illustrated in Figure 10. Initial data show that the reference cigarette caused greater depletion of GSH compared to the prototype THP.

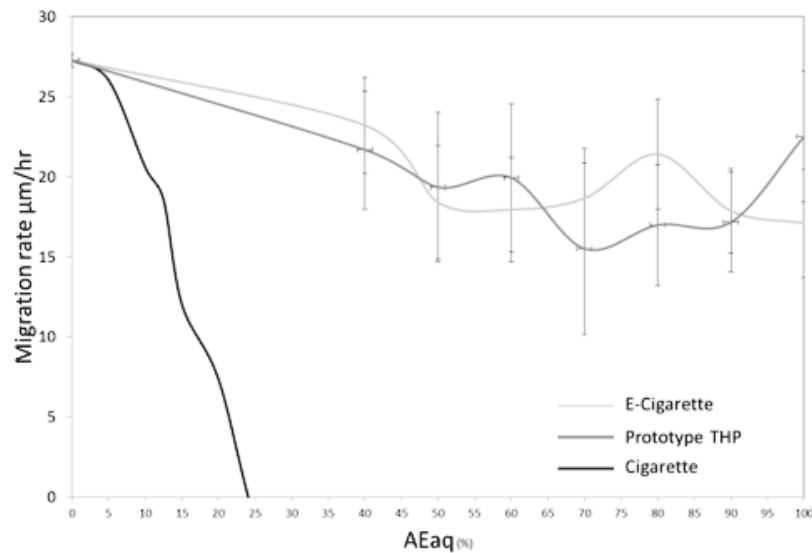
Figure 10. Oxidation of GSH to GSSG in lung epithelial cells when exposed to cigarette smoke and THP aerosol.



## 4.2 Cardiovascular Disease

Cigarette smoking is a well-described risk factor for cardiovascular disease (CVD). This is due at least partly to the tendency of smoke and smoke toxicants to promote atherosclerosis within the cardiovascular system [Unverdorben *et al*, 2009; Winkelmann *et al*, 2009]. Previously we have used an *in vitro* assay to model a number of the CVD endpoints to assess the risk reduction potential of products using endothelial cells (HUVECs) [Fearon 2012 and McQuillan 2015]. We assessed the ability of injured cells to migrate into and repair a mechanically induced wound in the presence of cigarette smoke and aerosols from a prototype THP and an e-cigarette (Figure 11).

Figure 11. Assessment of reference cigarette smoke and aerosol from a prototype THP and an e-cigarette using an endothelial cell migration assay



These data show that a mechanically induced wound repairs itself when exposed to aerosols from prototype THP and an e-cigarette, whereas when the cells are exposed to smoke from a reference cigarette the cells are unable to repair and re-establish endothelial integrity.

#### 4.3 Chronic Obstructive Pulmonary Disease

COPD is a major cause of morbidity and mortality worldwide (Rabe 2007), and is the result of chronic exposure to inhaled agents, such as cigarette smoke, noxious gases and particles, although over 90% of patients are reported to have a history of smoking (Mauders 2007).

To understand the mechanisms that underlie these key pathological processes and how exposure to tobacco smoke and aerosols from next-generation products drives the disease process, we are developing various *in vitro* models, including the use of primary cell types and 3-D reconstituted human tissue models *eg.* MucilAir™ [Baxter 2015] and EpiAirway™ [Neilson 2015]. The key events identified in the COPD AOP include mucus hypersecretion, impaired muco-ciliary clearance, fibrosis and lung tissue re-modelling.

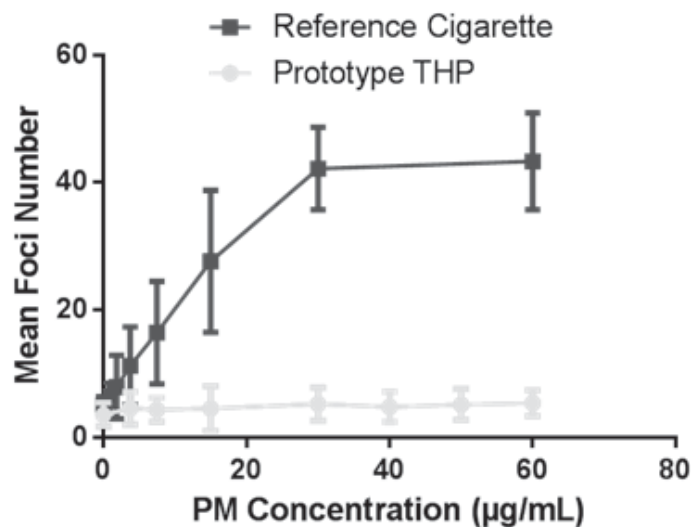
#### 4.4 Cancer

Cancer, a leading cause of death worldwide, accounted for 8.2 million (22%) of all deaths from non-communicable disease in 2012 [WHO 2012]. Lung cancer is by far the biggest cause of death, accounting for 22% of all cancer deaths in the UK in 2012 [Cancer Research UK 2014]. Cancer development is defined over three stages:

- 1) Initiation – irreversible changes to a cancer-related gene
- 2) Promotion – reversible selective clonal expansion of the initiated cell via growth stimulation or inhibition of apoptosis (programmed cell death)
- 3) Progression – stable alteration of genes in an initiated cell

We are investigating the development and application of *in vitro* models of cancer initiation (ie, DNA damage / mutation assays) and promotion (cell transformation assays) in the testing of the effects of cigarette smoke and toxicants. This research and our findings to date have shown that whole mainstream smoke aerosol induces oxidative DNA damage in lung cells (Comet assay) and that cigarette smoke particulate matter (PM) acts as a weak initiator and strong promoter *in vitro* (Bhas 42 cell transformation assay). We have further assessed promoter activity using the Bhas transformation assays using PM generated from cigarette smoke and a prototype THP as shown in Figure 12. PM from a reference cigarette was significantly positive at several concentrations tested in the Bhas 42 promoter protocol, whereas PM from the prototype THP was negative at all concentrations tested in the same range.

Figure 12. Assessment of reference cigarette smoke and aerosol from a prototype tobacco heating product using the Bhas 42 cell transformation assay



Cell transformation assays have been shown to be predictive of the carcinogenic potential of chemical and physical agents. In addition to the Bhas assay, the Syrian hamster embryo (SHE) cell transformation assay is one such system. We developed a two-stage SHE cell transformation assay that allowed the study of initiators and promoters in the carcinogenic process. The initiators and promoters tested in this assay were found to behave similarly to their reported *in vivo* characteristics. This

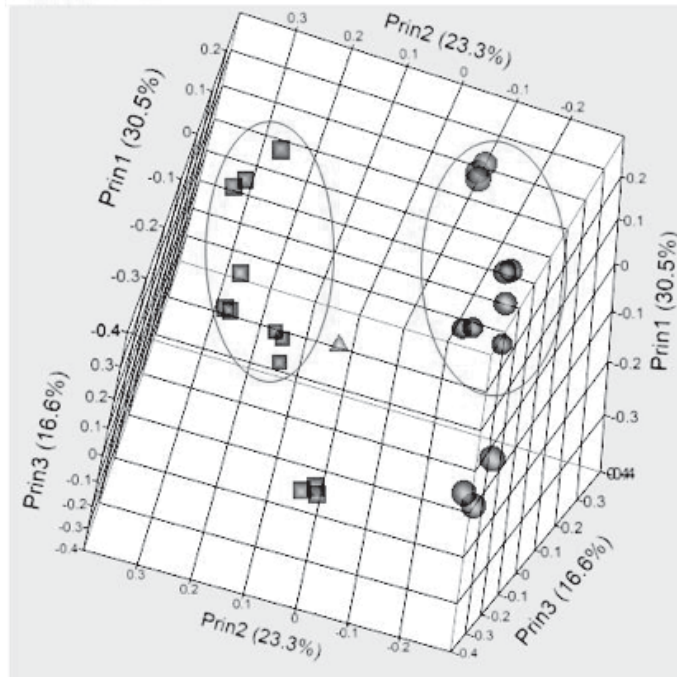
two-stage assay was also used to assess the activity of cigarette smoke PM, and was found to act at both stages of cell transformation [Breheny 2005]. A number of molecular markers were also identified that could provide both a mechanistic link to cell transformation, and a means to a possible replacement for morphological transformation as the endpoint of this assay.

#### 4.5 Integrating 'omic and *in vitro* data sets

Epidemiology is the gold-standard approach to determine the risk of disease associated with lifestyle and chemical exposure. Epidemiological studies, however, require a marketed product and are conducted in large populations over an extended period of time. Novel tobacco heating and nicotine products are either in development or emerging on the market thus, epidemiology is not suitable to inform regulatory decisions at this point in time. Furthermore, epidemiology is observational and does not provide a mechanistic understanding of the events leading to a disease. Alternative approaches are therefore required to assess the risk of tobacco heating and nicotine products that can inform regulatory decisions. British American Tobacco has publically stated that it will not conduct research on animals unless stipulated by a regulator and this *in vitro* approaches offer an alternative for product risk assessment provided that studies carefully consider (i) the route of exposure and dose, (ii) the relevance of cell type and metabolic competency, (iii) and the biological endpoints informative of toxic stress.

We have characterized one of those commercially available 3D airway epithelial models (MucilAir™) for metabolic competency (Figure 13) and demonstrated that key cytochromes, (CYPs), involved in the bioactivation of smoke toxicants were stably expressed for up to 6 months [Baxter 2015]. The ability to maintain differentiated metabolically competent cells for a prolonged period of time allows the conduct of both acute but also longer term chronic exposure studies.

Figure 13. Example of principal component analysis comparing the expression profile of 40 xenobiotic metabolism genes in MucilAir™, EpiAirway™, HBECs, NCI-H292 with and without TCDD induction in pooled human lung (smokers and non-smokers), (Baxter 2015)



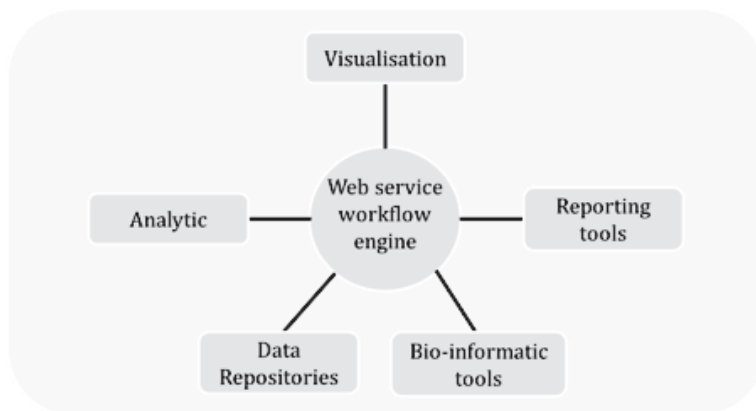
However, it is not likely that the events identified using classic toxicological endpoints comprehensively map the pathway to disease and possibly, key events could be missed. Yet, if a detailed signature of biological perturbations can be obtained and linked along a sequence over time and dose range they can be used to describe the causal relationship between exposure and disease. Such information supports the AOP approach. The integration of omics profiling with classical toxicology and exposure is an emerging approach. It combines the power of comprehensive screening of gene expression, proteins, and metabolites with the verification of single mechanistic and histological endpoints.

To expand on our previous example, transcriptomics, metabolomics and proteomics can be applied to cultures of respiratory epithelial cells exposed to reference cigarettes and tobacco heating and nicotine products. Associations between gene and protein expression and metabolic counterparts can be explored. If gene expression changes are associated with changes in the protein and the metabolic pathways they regulate, then the observation is likely to be biologically relevant and can be further supported by evidence such as change in histology, organelle structure and functions. Using gene ontology enrichment and knowledge-base bioinformatics tools including biological networks and disease networks curated from literature databases, the biological functions affected by these perturbations can be catalogued and the potential role in disease extrapolated.



Toxicological endpoints, such as cytotoxicity, oxidative stress and membrane integrity, will be included to support the associations between stress endpoints and pathway perturbations. To be able to work with omics data in an efficient way requires sophisticated computational techniques and infrastructures. A typical computational infrastructure required for bioinformatics where high storage capacity and processing power are essential is summarised in Figure 14.

Figure 14. Bioinformatics infrastructure for integrating large heterogeneous data sets



#### 4.6 Clinical Studies: Biomarkers of Exposure (BoE)

To assess whether pre-clinical reductions are observed and measureable in humans, we measure biomarkers in clinical settings. Biomarkers of interest are those of exposure (eg, external exposure and internal dose) and effect (eg, health impairment and early disease precursors) [Puntman 2009; Schmidt 2006].

Biomarkers of exposure (BoEs) offer the potential to measure exposure to smoke constituents and toxicants independent of individual smoking behaviour and can be integrated with pre-clinical data. Previously, we conducted BoE studies on subjects who switched from a conventional cigarette to an RTP cigarette, and showed significant reductions in toxicant yields versus those who remained smoking a conventional cigarette [Haswell 2014 and Shepperd 2015]. In addition to a previous review of BoEs for tobacco products assessment [Gregg 2013], the BoEs that were measured in the above studies that demonstrated utility for aligning with the chemistry measurements (conventional versus RTP cigarette) are proposed for assessing products across the risk spectrum and are listed in Table 2.

Table 2. Chemical constituents and corresponding Biomarkers of Exposure (BoE)

Smoke Constituent	BoE
Nicotine	Total nicotine equivalents (TNeq)
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)# *	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)
N'-nitrosonornicotine# *	N'-nitrosonornicotine
Pyrene	1-Hydroxy pyrene (1-OHP)
1-Amino naphthalene# *	1-Amino naphthalene# *
2-Amino naphthalene# *	2-Amino naphthalene# *
3-Aminobiphenyl# *	3-Aminobiphenyl# *
4-Aminobiphenyl# *	4-Aminobiphenyl# *
o-Toluidine	o-Toluidine
1,3-Butadiene# *	Mono-hydroxybutylmercapturic acid (MHBMA)
Benzene# *	S-phenylmercapturic acid (S-PMA)
Acrolein# *	S-(3-hydroxy-propyl)mercapturic acid (3-HPMA)
Crotonaldehyde# *	3-hydroxy-1-methylpropylmercapturic acid (HMPMA)
Acrylonitrile# *	2-Cyanoethylmercapturic acid (CEMA)
Carbon monoxide (CO)# *	Exhaled carbon monoxide (ExCO)/ Carboxyhaemoglobin (COHb)

#[Burns 2008]

\*[FDA 2012b]

## 5.0 Phase 3: Individual Risk Reduction

### 5.1 Clinical studies: Biomarkers of Biological Effect (BoBE)

The third phase of the assessment framework builds on the pre-clinical and BoE studies from phases 1 and 2 and focuses on measuring the impact of tobacco heating and nicotine products on human biomarkers of biological effect (BoBE) in a clinical setting. In addition, the integration of BoBE with *in vitro* endpoints is proposed to enable a comprehensive picture of the physiological response of subjects to a product.

Currently, we and others have used an array of BoBE to discriminate between smokers and non-smokers, which are informative of cardiovascular and

pulmonary stress [Haswell 2014]. We recently conducted a six month clinical assessment of an RTP cigarette using BoEs and BoBEs [Shepperd 2015]. The RTP cigarette demonstrated lower measured toxicant yields and subsequent BoEs versus a conventional cigarette, however, it did not manifest significant changes in BoBE to warrant further investigation for risk reduction potential. A number of potential BoBE that are proposed for the evaluation of products across the risk spectrum are summarised in Table 3.

*Table 3. Potential Biomarkers of Biological Effect (BoBE) for assessing the reduced risk potential of novel tobacco and nicotine products*

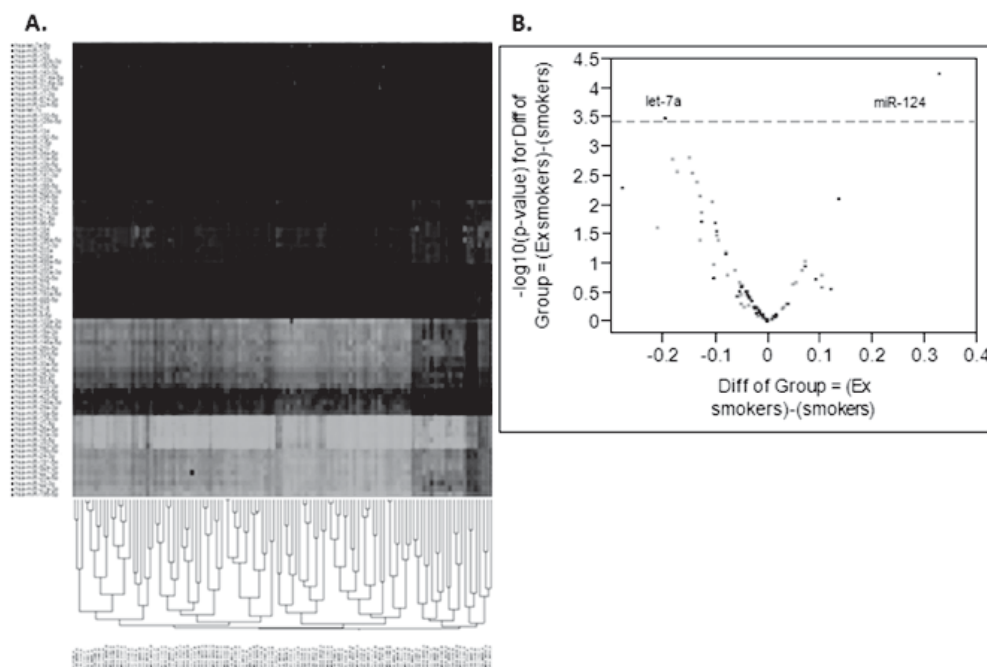
Biofluid	Biomarker of Biological Effect**	Justification	Reference
Urine	Thromboxane (11-DTX-B2)	Has shown significant difference between current and never smokers	Haswell 2014
	Prostaglandin (8-epi-PGF2 $\alpha$ ) Type III	Has shown significant difference between current vs ex-smokers and current vs never smokers	Haswell 2014
	Total NNAL	Exposure to nitrosamines has been described as predictor of respiratory cancers.	Yuan 2011
	Micro-RNA panel	Multiple evidence of association of circulating miRNA with diseases and early diagnosis of lung cancer. Further work needed to validate as predictor of disease	Wozniak 2015
Serum	HDL Cholesterol (HDL-C)	Has shown significant difference between current vs ex-smokers and current vs never smokers	Haswell 2014
	InterCellular Adhesion Molecule (sICAM-1)	Has shown significant difference in smokers of the prototype RTP when compared to baseline was compared at end of study	Shepperd 2015
Blood	White blood cell total count	Has shown significant difference between current vs ex-smokers and current vs never smokers	Haswell 2014

## 5.2 Integration of 'omic and biomarker data

Biomarkers of Biological Effect (BoBE) have utility for the assessment of individual risk reduction with products across the risk continuum. However, looking at each BoBE in isolation does not give a comprehensive picture of the physiological

response of subjects exposed to a product. Furthermore the use of an array of biomarkers developed in the context of combustible product testing can miss adverse events potentially indicative of toxicity caused by tobacco heating and nicotine products. Toxic insults lead to tissue damage with secretion and leakage of cellular material in biofluids that can be quantified in serum, sputum, saliva, and urine with the added advantage of minimizing the need for invasive sample collection procedures. Multiplatform metabolomics, epigenetics and proteomics analyses allow the quantification of thousands of metabolites, proteins, and miRNA in biofluids and discovery and qualification of BoBE. The use of multivariate statistical analyses of “global perturbation” of metabolites, proteins, and miRNA has the potential to extend understanding of product risk. For instance, in recent metabolomic studies we and others have clearly highlighted that smoking alters metabolites that are part of the glutathione pathway, a well-known antioxidant [Garcia Perez]. In a screen of plasma miRNAs we identified changes in the level of two tumour suppressors [Banerjee 2015] (Figure 15) in healthy smokers associated with human lung tumours prognosis [Berghmans *et al*, 2013] of which one was also correlated with a BoE to a smoke toxicant.

Figure 15. Example of epigenetic miRNA profile from smokers, non-smokers and ex-smokers (adapted from [Banerjee 2015])



It is important to note however that unless retrospective clinical studies are conducted biological pathway alterations and BoBE cannot be fully validated as disease predictors. Nevertheless they are informative about changes in body homeostasis and identify events in an AOP potentially on the path towards a disease.

In the context of risk assessment of products across the risk continuum, comparisons can be performed with smokers and non-smokers using a multiplatform approach combining proteomics, metabolomics, epigenetics, and known clinical BoBE. Correlations between exposure, biological effect (BoBE and 'Omics) and biological pathway alteration identified using knowledge-based tools such as IPA Ingenuity® will provide a robust multi-evidence approach to product risk assessment. In order to link *in vitro* data with clinical biofluid data we propose to perform omic screens to characterize the secretome of *in vitro* models exposed to tobacco products. Since matrices such as blood and plasma are the information superhighway regarding body homeostasis, biomolecules secreted in blood by damaged or stressed tissues can be quantified and compared with those secreted in media by *in vitro* models. Limited animal studies allowing tissue biopsies and blood collection might be needed to bridge *in vitro* data with clinical data. Finally, we will also maintain the development of classic single BoBE endpoints, in line with the literature, especially those proven to be predictive of disease.

## 6.0 Population Risk Reduction

The fourth phase builds on phases 1-3 and lays out an approach describing a range of population studies that would enable the assessment of the risk potential of products across the risk spectrum at a population level. This is important because an understanding of whether differences in risk estimates translate to effects at the population level is required. Risk at the population level is constituted by two main factors:

- Health risk specific to the product
- Expected behaviour of potential consumers

Currently, health risk is often determined subjectively, for example by a panel of experts extrapolating from biological studies [Nutt *et al*, 2014]. Behaviour of potential consumers might be affected by personal perceptions, for instance that the product *eg.* a tobacco heating or nicotine product is much safer than cigarettes and that could potentially lead to never-smokers starting to use these products and ex-smokers returning to tobacco/nicotine use. At the population level, these behavioural changes might have a negative impact for some disease endpoints.

We propose the monitoring of the effects of launching a tobacco heating or nicotine product through central location tests and focus groups. Perception attributes, such as attractiveness and health risk perception, are monitored through home use testing and clinical studies.

Population modelling techniques are well established and can be used to gather information that aids prediction of outcomes for endpoints of interest. For example, epidemiological data show that mortality rates depend on smoking status (often categorised by age and gender). Prevalence of smoking statuses based on historical

data can be used to estimate the number of deaths in smokers relative to those in never smokers. This scenario, commonly known as the *status quo* scenario, assumes that nothing changes. In a counter-factual scenario, a product with a high risk reduction potential *eg.* a tobacco heating or nicotine product would be introduced to a market and is widely adopted by smokers, which leads to reduced mortality because the probability of survival increases.

### 6.1 Risk perception

Some regulators, including the FDA, are proposing to make the assessment of population level risks part of the regulatory process [FDA, 2012b]. Although the required population level tests have not yet been fully defined, we anticipate that they will include studies examining risk perception, uptake, impact of marketing and other information and level of risk imparted to individuals by use of the product alone or in combination with other tobacco or nicotine products. Data will need to be collected from current and non-users in qualitative and quantitative studies, and before and after a new product is marketed and computation models will be needed to estimate the effects.

### 6.2 Abuse liability

Assessment of the potential for abuse liability (also termed abuse potential) will feature prominently in any assessment of population-level effects of a novel product. In the pharmaceutical industry, abuse liability is a well-defined concept. The FDA, for example, uses the definition “the use of a drug in nonmedical situations, repeatedly or even sporadically, for the positive psychoactive effects it produces” [FDA, 2010]. Examples of such psychoactive effects include sedation, euphoria, perceptual and other cognitive distortions, hallucinations, and mood changes. Drugs with abuse potential often, (but not always) produce psychic or physical dependence and might lead to addiction.

Translating this concept to the context of risk reduction through the switching to products across the risk continuum and given suggestions by Carter *et al.*, [Carter 2009] amongst others, we define abuse liability as “The potential for a nicotine-containing product to create dependence behaviours and promote compulsive self-administration with negative consequences of use.” Physical dependence is characterised by the development of tolerance to tobacco product and/or the onset of withdrawal symptoms upon stopping use. Psychological dependence is characterised by persistent tobacco-seeking and tobacco-use behaviours, impairment in behavioural control and craving, and inability to abstain consistently. Currently, while no specific guidance exists for assessment of abuse liability for products across the risk continuum we aim to include such assessments in clinical studies by examining the following features:

1. Nicotine uptake in pharmacokinetic studies of healthy smokers, under defined and *ad libitum* puffing conditions and following overnight

- abstention, and subjective assessments of constructs such as craving relief, satisfaction and intent to use again;
2. How the novel product affects symptoms of withdrawal, using standard scales (eg., the Minnesota Nicotine Withdrawal Scale) in abstinent smokers;
  3. The degree to which a subject is willing to pay or work to “earn” use of the novel product. Such behavioural economic assessment could allow cross-sectional examination of withdrawal symptoms and “value” of allowing product use in accustomed, abstaining users of different types of products.

Abuse liability assessment of novel reduced risk tobacco and nicotine products should include the potential for uptake by non-users, relapse in ex-users or dual use by current smokers. Non-users and ex-users, however, are difficult to study for ethical reasons. As such our view is that post-market data of real-world use patterns will be key to obtaining such data.

### **6.3 Consumer usage data**

Examination of consumer usage patterns is crucial to discerning population-level effects of novel products. Factors that might impact population risks relate to product usage patterns (eg, puffing topography, number of uses per day, duration of uses etc) and longer-term behavioural effects (eg, product uptake and use of other tobacco products).

The FDA advises that scientific studies submitted by applicants should inform the evaluation of the likelihood that current tobacco product users will start using the product; that those who adopt the product will switch to another harmful product (including their original product); that consumers will use the product in conjunction with other tobacco products; that users who may have otherwise quit will instead use the product; and that consumers will use the product as intended or designed [FDA, 2012a].

Data collection on consumer use-behaviour should be performed before and after launch. Pre-market studies must assess consumer interactions with the product, usage patterns, inhalation depth and frequency, patterns of co-use etc., in controlled and real world settings. Small group assessments done with validated questionnaires might also be useful and can include non-users of tobacco products. Post-market surveillance studies could assess long-term behavioural and health effects.

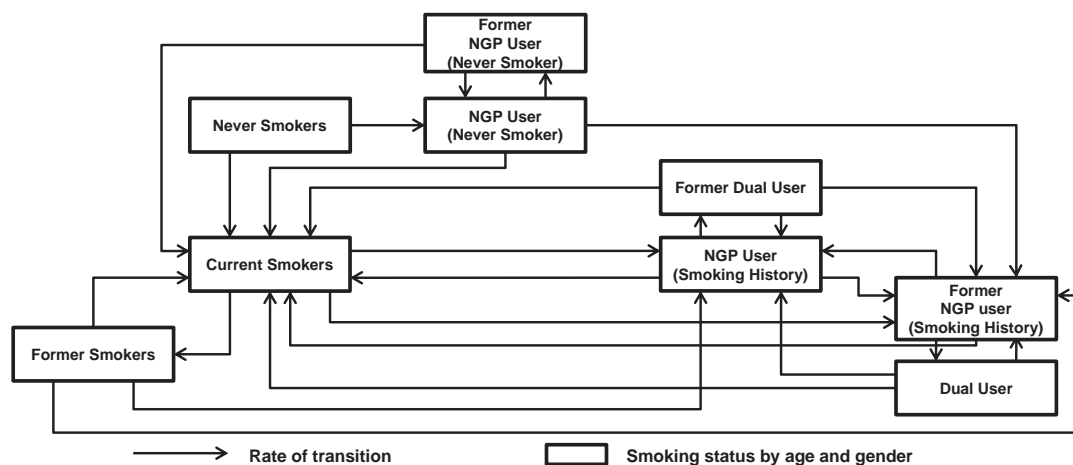
### **6.4 Population modelling**

There are many approaches to computational modelling but within the context of tobacco regulation they tend to be classified in three main categories: statistical modelling, agent-based modelling and system dynamics approaches. An approach is proposed based on a compartmental model using system dynamics, which aligns with previous models used by regulators.

Population models use the same type of inputs, *i.e.* relative risk (RR) between different smoking statuses and measures of prevalence for each type of smoking status. Relative risks for never, former and current smokers will be calculated from epidemiological data while RRs for the new product will be based on multiple assessments as described in phases 1-3 of our proposed product assessment framework. Prevalence data for conventional cigarettes can be extracted from public sources and models may take into account initiation rates as well as relapsing rates from former to current smokers. Uptake, prevalence and transition rates between a new product and the other potential states need to be estimated using perception studies, intention to use and other tailored studies to try to predict consumption patterns and behaviours.

We propose a compartmental system dynamics model which represents a population from an initial year and is then updated every year using births, deaths and migration rates to provide long term projections. Compartments are divided by age and gender categories and it tracks time since quitting for former smokers. Compartments represent all possible combinations of smoking statuses, including dual use (see Figure 16).

Figure 16. System dynamics model illustrating the introduction of a novel reduced risk tobacco or nicotine product in a market.



In system dynamics, “what if” comparisons allow investigation of the impact of modifying or adding new parameters in the model. These comparisons are made possible by using a status quo scenario as a benchmark to judge whether the change under investigation is likely to have a positive or negative effect in the population and how that effect may change with time. For the purpose of assessing the potential of reducing population risk with products across the risk continuum, the differences in health outcome projections are investigated using a market that follows current trends compared to one in which a new product is launched, and hypothetically those trends are changed. These changes are not



only due to the intrinsic relative risk of the product but also to changes in smoking statuses prevalence from different segments of the population and alteration of their consumption patterns.

It is important to keep in mind during the whole model development process that population models are only as good as the parameters used to create them. Models must provide a balance between simplicity and realism and the logic should be intuitive and not a 'black-box' for the end user. Therefore, it is essential to be able to justify the model decisions, assumptions and the chosen parameters in a transparent way.

### **6.5 Post-market surveillance (PMS)**

This framework proposes a series of pre-clinical, clinical and population studies on products across the risk continuum before launch to ensure that risks are minimised. However, once products are in general use, post-market surveillance could be carried out to identify unintended consequences and unexpected disease outcomes. Data collected during post-market surveillance would inform any likely intervention by either the manufacturer or the regulator in the interest of public health. This could include both passive surveillance, which relies on data reported spontaneously by consumers and healthcare professionals, and active surveillance data collected through intervention and epidemiological studies and population-wide surveillance.

A PMS programme could include information about product usage patterns, consumer perception; provide data with respect to the health risks, and the effect on morbidity and mortality as compared to using other products or quitting use of tobacco products. Specific information could also be collected such as health care visits, physiological measurements and adverse events.

PMS will play an important role in monitoring and re-evaluation in the case of a regulatory claim being approved and will need to be designed differently for reduced exposure and reduced risk claims. These activities are needed to monitor post-launch product changes and consequent effects on the population to ensure consumer safety and regulatory compliance.

### **7.0 Conclusion**

In this paper a scientific framework is proposed to assess the risk reduction potential of novel tobacco and nicotine products across the risk continuum. This four-phase approach comprises stewardship science, exposure reduction, individual risk reduction and population risk reduction and encompasses a range of pre-clinical, clinical and population studies which would enable assessment at both the individual and population levels.

The AOP model is incorporated in this framework as it has the potential to map key events from toxicant exposure through to smoking related diseases using a variety of chemical, *in vitro*, 'omic and biomarker studies. An additional benefit to the proposed framework is the possibility of integrating large and heterogeneous data sets by the combined methods of data acquisition, data processing, bioinformatics and toxicology, and thereby enabling the ranking of risk of products across the risk continuum.

Some of the challenges of this approach will be the harmonisation of approaches, agreement of methodologies and standardisation across the various studies. Transparency is key and would be facilitated through the publication of data and making datasets publically available. The advent of the workshops moderated by independent groups such as the Institute for *In Vitro* Sciences (IIVS), is an example of how regulatory, public health, academia and industry scientists could work together to agree and harmonise on a science framework, which could be used for evidence based regulation of products across the risk continuum.

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