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by scientists for scientists
This is our first Science and Technology Report. It provides an overview of our research programmes, which are focused on using world-class science to develop and assess novel technologies and to create safer tobacco and nicotine products. The work is done primarily by our in-house scientists based in two UK locations (Southampton and Cambridge), but we also collaborate with science and technology partners around the world.

Our Group Research and Development (GR&D) operates in a competitive sector, and our primary focus is to develop and leverage our science to provide our consumers with innovative products that are superior to those of our competitors. Nevertheless, given the enormous negative impact that cigarette smoking has on public health, we have long believed that there is an urgent need to develop products that reduce this impact, and that the building of the emerging science base needed to evaluate these products should be non-competitive. We also believe that consumers should have access to meaningful product information based on sound science. We understand that we cannot reduce the public-health impact of cigarette smoking by ourselves. Thus, we wish to work more with policy makers, regulators and public-health advocates to create a supportive environment for the development of safer tobacco and nicotine products and to help consumers make informed decisions about such products. Our aim is to develop the best consumer-led products possible based on robust science and make a significant contribution to developing the scientific frameworks that will enable their objective evaluation.

We also believe that the time has come to further develop tobacco and nicotine science with new participants free from the debilitating politics of the ‘tobacco wars’ of years past. We are pleased to open our doors to interested visitors, to host on-site scientific conferences, to seek collaborations with academic partners under US National Institutes of Health (NIH) grant schemes, to publish in peer-reviewed journals, to present at key scientific conferences and to provide overviews of our goals and achievements on the company website.

The thought leader in the regulation of tobacco and nicotine products is currently the US Food and Drug Administration (US FDA), which, encouraged by reports from the independent US Institute of Medicine, is setting out frameworks for evaluation of Modified Risk Tobacco Products (MRTPs). The US FDA is developing its own scientific resources at the Center for Tobacco Products, growing networks with other US scientific agencies and disbursing a large programme of grants based on research priorities to improve the quality of the science underpinning tobacco product regulation. Much of the research in this report seeks to fill gaps in knowledge described by the Institute of Medicine and US FDA.

The purpose of this report is not only to present the science we and our partners are undertaking, but also to explain why the research is important and where we expect it to take us over the next few years. We plan to provide an update every two years, but interim progress reports will be posted more frequently on our website, www.bat-science.com. We believe that progress is accelerated through dialogue and collaboration, and so your comments are always welcome.
Cigarette smoking is a leading cause of various fatal diseases, including lung cancer, chronic obstructive pulmonary disease (COPD) and cardiovascular diseases (CVDs). The tobacco-related increases in risk for such disease are dose-related, as demonstrated by many epidemiological studies, and reduce on quitting. Tobacco smoke is a complex mixture that contains many known toxicants. The range of risks varies with different tobacco and nicotine products, with medically regulated pharmaceutical nicotine-replacement products generally regarded as the least risky and cigarettes the most risky. Cigarettes, however, are in most countries by far the dominant and most popular form of tobacco product.

In 2001, the US Institute of Medicine published its report, *Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction*. It introduced the concept of Modified Risk Tobacco Products (MRTPs, originally termed “Potential Reduced Exposure Products” or PREPs). The report set out an assessment framework that includes the need for regulatory governance and which has evolved over time with the changing science. Taking this and a subsequent Institute of Medicine report, the US FDA has developed draft guidelines for the assessment of MRTP applications. For the past 10 years, our research has followed a similar approach to that set out by FDA. We have used prototype cigarettes with novel technologies to reduce toxicant yields to help develop and test the scientific framework for assessing the potential for novel products to reduce exposure to tobacco smoke toxicants and to assess whether any measured reduction might be meaningful in terms of reducing the health risks of tobacco consumption.

Although the development of specific technologies and products is competitive, we believe that the underlying science needed to assess MRTPs should be non-competitive, not least because in some areas it is particularly challenging. With this approach in mind, we have presented details of our research programmes to the US FDA, extensively published our research and actively sought to collaborate with other groups, although the latter objective remains challenging within both the industry and some spheres of academia. We are actively seeking NIH/US FDA grants for research with various partnerships where we would not financially benefit but could supply products or expertise to the projects.

Our current science and technology priorities are:

» Continue to discover and develop for commercial use toxicant-reducing technologies and next-generation tobacco and nicotine products that can be characterised for their potential to reduce health risks as compared to those conferred by current tobacco products

» Develop methodologies for chemical characterisation that will enable measurement of toxicants in a range of different style of tobacco and nicotine products

» Use aerosol science to predict deposition of product emissions in the human respiratory tract

» Develop computational toxicology models and non-animal experimental approaches to characterise key toxicants, assess their interactions with other toxicants and to generate dose–response models for various disease-related pathologies

» Use the latest advances in molecular biology and toxicology to develop a range of *in vitro* models to assess emissions from novel products and give insights into their contributions to disease-related processes

» Develop methodologies to investigate and measure biomarkers of exposure, effective dose and biological effect, to provide meaningful predictors of health risks with qualification in clinical studies

» Undertake research into the scientific characterisation of e-cigarettes

» Define good research practice for all the research at GR&D

This report sets out recent progress in each of these areas and highlights future directions, challenges and opportunities.

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**DR CHRISTOPHER PROCTOR**

Chief Scientific Officer

Chris was educated as a mass spectrometrist and his training included postdoctoral research at Cornell University under a Fulbright Scholarship. He has published widely in the field of tobacco science and is the author of a book that focused on historic approaches to tobacco harm reduction.
Reducing exposure to toxicants: the latest technologies

We strive to create new technologies and satisfying cigarette substitutes with the potential to substantially reduce the health risks to consumers of currently available tobacco products.

We employ an integrated science and technology process where new scientific findings (for example, on priority toxicants) drive new technology development, and new technology drives the need to develop new methods of risk characterisation.

For our next-generation heat-not-burn products, we are looking to generate a sufficiently satisfying nicotine aerosol to make these products a substitute for cigarettes that is reasonably acceptable to consumers.
Creating innovative tobacco products that substantially reduce toxicant exposure as compared to current products, and testing their potential for reducing risk of tobacco-related disease, is the focus of our state-of-the-art R&D facilities and the passion of the scientists who work there.

Epidemiology consistently shows dose–response relationships between cigarette smoking and a variety of diseases; in addition, cessation of smoking is associated with reduction of the increased risks of disease conferred by tobacco use. These data form the basis for a seminal 2001 US Institute of Medicine report that introduced the concept of potential reduced exposure products or PREPs, now known as MRTPs. Such products, it suggested, might have the potential to reduce current health risks associated with tobacco use.

For some years we have been investigating whether it might be possible to make cigarettes less risky by applying reduced toxicant technologies to prototype cigarettes and testing them in chemical, biological and clinical studies.

Ideally, the identity and the dose–response relationships of the precise toxicants responsible for smoking-related diseases will be characterised, meaning that risk reduction would simply be a matter of developing new technology and testing frameworks to demonstrate that the technologies resulted in substantially reduced exposure to these toxicants. Unfortunately, there remains considerable scientific uncertainty regarding which toxicants in cigarette smoke need to be reduced and the level of such reductions necessary to produce cigarettes that can be proven less risky to health. Because of this, we have taken a pragmatic approach of developing and testing various technologies to reduce a wide range of toxicants, despite the reality that, to date, we are unable to substantially reduce all of the harmful and potentially harmful toxicants identified.

Cigarette smoke includes particles formed during the combustion of the tobacco, and it is very difficult to selectively reduce toxicants in the particulate phase once it is formed. Rather, the toxicants (or the precursors to the toxicants) are ideally reduced in the tobacco prior to combustion. Our research group at Cambridge is focusing on biotechnology to reduce a range of tobacco toxicants, including tobacco-specific nitrosamines and heavy metals such as cadmium in tobacco plants.

It may be possible to produce small health risk reductions by modifying cigarettes with such reduced toxicant technologies, but for substantial reductions it is likely that we will need to have a step change in toxicant exposure, by, for example, limiting toxicant formation in the first place.

We are working on novel products where the tobacco is not burned. In these heat-not-burn products, the tobacco is brought to a temperature well below that found in combustion through a variety of sophisticated heating systems. Doing this produces an aerosol containing nicotine and compounds that provide the consumer with taste and flavour, but with far fewer toxicants than are present in cigarette smoke.

E-cigarettes are another example of a product in which a much simpler aerosol containing nicotine is inhaled (nicotine is typically extracted from tobacco).

We have also made progress in developing oral tobacco that offers reduced health risks, as seen in many epidemiological studies, in comparison both to cigarette and traditional chewing tobacco. Snus, a low-toxicant smokeless oral tobacco product used for over 100 years in Sweden, has been proven to be substantially less risky than smoking cigarettes, although we have found it difficult to make snus an acceptable substitute for smokers in countries where oral tobacco use is not common.

Recent successes

We have developed four technologies that have been incorporated into a series of reduced toxicant prototype cigarettes. These technologies are expected to lead to reduced levels of toxicants in the smoke: first, a treated tobacco processed to remove some of the pre-cursors to smoke toxicants; second, a glycerol-containing tobacco substitute; third, a novel nano-porous carbon that is very efficient at adsorbing many volatile and some semi-volatile toxicants such as 1,3-butadiene and benzene; and, fourth, an ion-exchange resin that is effective at selectively reducing some aldehydes and...
hydrogen cyanide. The first two technologies are applied to the tobacco rod and the latter two are applied to the filter; they have been combined in different ways to produce prototype cigarettes. Tests in the laboratory with smoking machines showed that the prototypes yielded substantially reduced levels of various tobacco smoke toxicants compared with conventional cigarettes. We have conducted and published the results of a 6-week clinical study demonstrating that the reductions in certain toxicant levels found in chemistry studies translate into reductions in biomarkers of exposures for those toxicants in groups of smokers switched to reduced toxicant prototype cigarettes. We have also completed, and are currently analysing, results from a 6-month clinical study that included biomarkers of biological effect.

We have also made good progress on our heat-not-burn prototypes and begun characterising their potential to reduce health risks and act as substitutes for cigarettes. Nicoventures (our separate nicotine business) is currently developing and marketing a range of e-cigarettes.

**Aims for the next two years**

- Develop processes to scale existing toxicant-reducing technologies
- Continue a plant breeding programme aimed at reducing toxicants in tobacco leaf
- Investigate new technologies to reduce toxicants that are currently difficult to reduce, including benzo(a)pyrene, 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNK) and carbon monoxide
- Develop, test and fully characterise next generation heat-not-burn products capable of reducing toxicant yields to a greater extent than is achievable in cigarettes
- Nicoventures will develop and bring to market improved nicotine products

**Challenges requiring collaboration**

There remain substantial scientific opportunities for collaborative research aimed at identifying the most important toxicants and their dose–response relationship to various diseases. This is covered in other sections of this report. In addition, we hope to accelerate our efforts in the following areas through technology partnerships: developing technology to address toxicant precursors in leaf; processing of tobacco to reduce levels of toxicants and their precursors; designing new adsorbent technologies to selectively reduce volatile toxicants in smoke; and, developing next-generation heat-not-burn products.
Chemical characterisation

The development of standardised methods for the reporting of smoke and tobacco toxicants is a complex technical challenge. Standardised methods ensure consistent results are produced in our laboratories, resulting in optimal use of resources and better support for engagement with external stakeholders concerned with the measurement of toxicants for regulatory purposes.

The chemical characterisation of next-generation products is essential to their development, and our teams interact strongly to ensure that such measurements are relevant to consumer use of the products.
Chemical characterisation of tobacco smoke and next-generation products is an important starting point for assessing the health risks associated with various products.

Both tobacco and tobacco smoke are complex matrices and present interesting analytical challenges.

Historically, a list of 44 smoke toxicants (often known as the 'Hoffmann list' after American Health Foundation scientist Dietrich Hoffmann) was used to characterise the range of likely toxic constituents in cigarette smoke. Over the years, these toxicants have been measured in cigarette smoke using many different analytical methods. The methods used by GR&D are consistent with industry best practices such as Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) standards and inter-laboratory ring trials, but have not been harmonised or internationally validated. Consequently, inter-laboratory differences in measurements range from four to ten times the optimum precision, potentially introducing bias in results.

More recently, the US FDA has identified 93 harmful and potentially harmful constituents (HPHCs) in tobacco and tobacco smoke. The US FDA’s Center for Tobacco Products has held workshops seeking information on tobacco analysis, to which we contributed. The Center for Tobacco Products is also seeking to generate new reference materials that may help substantially in the development of analytical methods.

The large number of cigarette smoke analytes believed to contribute or potentially contribute to tobacco-related disease means that new approaches need to be taken to multi-analyte assessment. We are exploring a range of such techniques, including nuclear magnetic resonance (NMR) and high-resolution time-of-flight mass spectrometry (TOF-MS).

Analytical chemistry also is essential in the characterisation of next-generation products such as heat-not-burn tobacco products and e-cigarettes. For these products, methods are needed to quantify much lower levels of toxicants, often in a simpler matrix than cigarette smoke. These methods must also comprehensively identify and characterise other constituents of emissions from these products that could, potentially, be different to those traditionally measured in cigarette smoke.

Standardised sampling and smoking procedures are well-defined for cigarettes and are set out in International Standards Organisation (ISO) analytical standards. This is not true for next generation devices. For these products, scientific studies are needed to identify the range of ways in which the products might be used, and analytical sampling techniques must be developed so that the aerosol generation and collection is relevant to human exposure.

Recent successes

Our analytical team is working co-operatively with the regional product centre for the Americas, based in Brazil, to evaluate core methods for the measurement of the 18 toxicants identified in the World Health Organization (WHO) Framework Convention on Tobacco Control. After optimisation of their statistical performance, the methods will be implemented in the European regional product centre in Germany, and the agreement of results between laboratories will be evaluated. Core methods for the determination of benzo(a)pyrene and tobacco-specific nitrosamines (TSNAs) in mainstream cigarette smoke have been implemented and are being statistically assessed.

Aims for the next two years

» Agree and publish a single definitive method for measuring each individual toxicant and class of toxicants, initially for the WHO priority toxicants but eventually for all of the US FDA’s HPHCs

» Agree the format and output for each method

» Establish the consistent use of the new methods across all of our analytical centres

» Publish methods for both targeted and non-targeted analysis of the emissions from next-generation products and e-cigarettes

Challenges requiring collaboration

Fit-for-purpose analytical methods need a considerable amount of collaboration, and as the list of potentially regulated tobacco smoke toxicants grows, so does the need for inter-laboratory studies. Additionally, the development of additional reference materials and products is important, and we will seek to contribute to current US FDA efforts in this area.

The development of next-generation products and technologies will require a range of measurement capabilities, including exploratory analyses (e.g. identifying...
all substances present), rapid semi-quantitative comparisons of samples (to understand differences in chemical profiles quickly) and quantitative analyses of established toxicants (to regulatory standards but preferably in a shorter time). To establish these capabilities we have invested in TOF-MS-based instruments and have investigated, through collaboration, the potential of NMR spectroscopy to identify and measure a wider range of product emissions. These instruments can simultaneously detect about 2,500 substances in mainstream smoke condensate. NMR spectroscopy can detect and measure 20 of the 44 Hoffmann toxicants in mainstream tobacco smoke condensate with a simple smoke-collection procedure, which is likely to be extended. We have also applied gas chromatography (GC) coupled with high-resolution TOF-MS to the comparative analysis of smoke samples. This method can automatically detect differences in chemical profiles and quantify them for potential risk assessment.

We also aim to develop a chemical informatics platform that will conduct rapid processing of multiple sample replicates, enabling correlation of chemical profile data between linked samples (e.g. green leaf, cured leaf, cut rag and smoke from the same sample). In conjunction, we will develop chemical screening techniques, such as rapid GC/TOF-MS screening of semi-volatile and vapour-phase contents and emissions, and characterisation of labile or non-volatile contents and emissions by high-performance liquid chromatography/TOF-MS.

Figure 1: Harmful and potentially harmful constituents of tobacco smoke as depicted by the Framework Convention on Tobacco Control, Health Canada and the US Federal Drug Administration.
Quantifying and assessing risk

A better understanding of the role and dose-response curves of toxicant families in tobacco smoke will help focus the technologies for reducing toxicants and guide the development of next-generation products.

Additionally, in January 2012, the US FDA outlined seven priority research areas relating to tobacco products. The third focused on reducing the toxic effects and carcinogenicity of tobacco products and smoke. The need for better science in this area is driven by regulators, whose future frameworks might be aimed at monitoring or lowering specific toxicant levels.
We are seeking to develop technologies to reduce the levels of individual constituents and/or groups of constituents in smoke. The aim of the Computational Toxicology and Drivers programme is to characterise and prioritise the contribution of individual and specific groups of tobacco smoke toxicants to the adverse health effects associated with smoking. To achieve this goal, we are combining predictive and experimental models.

The programme was set up in response to the idea that quantitative risk assessment methodologies could be applied to tobacco smoke toxicants. Using this approach to prioritise constituents for research may help make the efforts to develop reduced risk tobacco products more effective. One of the most influential publications in this area, by Fowles and Dybing in 2003 (Tobacco Control 12:242), described calculations conducted to prioritise the hazards for 158 chemical constituents in tobacco smoke. We therefore decided to investigate other possible quantitative risk-assessment paradigms that might be applicable to these and other tobacco smoke toxicants. The intention is to develop adaptable methodologies that can be incorporated into toxicological evaluations in various areas of our work, such as the development of novel products.

Our current risk-assessment paradigm is based on a combination of computer modelling approaches: margin of exposure calculations, mode of action reviews and physiologically based pharmacokinetic (PBPK) modelling. These models are supplemented with data from in vitro models of disease and conventional in vitro toxicology assays. The data can be used to generate margins of exposure where in vivo data are unavailable, and to provide support for the postulated modes of action for specific chemicals. In addition, it will be used to further refine our PBPK modelling tools. These activities will improve our understanding of the effect of mandatory toxicant reductions, assist with the prioritisation of research, provide data to facilitate product development and safety assessment, and facilitate identification of other important toxicants within tobacco smoke.

In the context of heat-not-burn products (which have far simpler aerosols and far fewer toxicants than tobacco smoke, but are still likely to have some level of some toxicants), the research will help put some perspective on the possible biological impact of the products.

Recent successes
In 2011 we published a paper outlining our use of margins of exposure to segregate tobacco smoke toxicants and prioritise our research (Food and Chemical Toxicology, 49:2921). This was followed in 2012 by a presentation at the Tobacco Science Research Conference, the proceedings of which were published in Recent Advances in Tobacco Science. The paper proposed categorising tobacco smoke toxicants according to the calculated margin of exposure and ascribing categories for toxicants for which data are limited or insufficient to generate a margin of exposure. Finally, we described the concept of calculating cumulative margins of exposure for groups of toxicants with similar toxicological properties, illustrated with aldehydes present in tobacco smoke.

The WHO Study Group on Tobacco Product Regulation has proposed a list of 18 tobacco smoke toxicants for reduction and monitoring. Some of these toxicants are found in the vapour phase of tobacco smoke, others into the particulate phase, with the remainder present in both phases. We have recently completed an assessment of all particulate phase toxicants present on the WHO list using our suite of genotoxicity tests, which includes in vitro micronucleus, Ames and mouse lymphoma assays. We have also evaluated a number of these particulate phase toxicants using our in vitro models of disease. In 2012, we developed gaseous exposure methods and applied these to ethylene oxide (a prototypical gaseous mutagen) in Ames mutagenicity and neutral red uptake cytotoxicity assays. This methodology is currently being
used to assess the remainder of the WHO toxicant list using the appropriate \textit{in vitro} genotoxicity tests and disease models.

**Aims for the next 2 years**

- Generate and continue to refine margins of exposure where possible for all tobacco smoke toxicants of interest
- Continue to produce peer-reviewed modes of action, going beyond WHO list of 18 toxicants to include additional chemicals on the longer US FDA list of harmful and potentially harmful constituents in tobacco smoke
- Set up a gaseous exposure system within GR&D to enable testing of gaseous tobacco smoke toxicants in our \textit{in vitro} models of disease
- Use the mode-of-action reviews to direct the assessment of individual tobacco smoke toxicants within relevant \textit{in vitro} models of disease and/or traditional genetic toxicity assays
- Develop toxicokinetic and PBPK, where possible, for all the 18 WHO Study Group on Tobacco Product Regulation chemicals and nicotine

**Challenges requiring collaboration**

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 the toxicologically active chemical species. To address this, we are developing PBPK models where possible for individual tobacco smoke toxicants. The output from these models might allow us to determine if the concentrations that give a positive biological response \textit{in vitro} are relevant to the human target-tissue dose. Although we have made some notable advances in developing experimental methods for \textit{in vitro} toxicant exposure and in the application of biologically based risk assessment, effective collaboration with external laboratories and experts dealing with tobacco and non-tobacco products would greatly aid these endeavours. Only through the combined and concerted effort of the tobacco and chemical industries, government agencies, contract research organisations and academia will significant progress be made in this area.

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**Figure 1:** Quantitative risk assessment methodologies applied to tobacco smoke in GR&D.
Aerosol science and dosimetry

Determining the appropriate and reproducible measurement of dose is an important step in analysing the toxicological action of smoke at cellular, organ and whole-body levels. Data sources, however, are currently highly fragmented as to such measurements, making it difficult to gauge how the delivery and action of smoke constituents in laboratory-based studies align with effects in human smokers. We have focused on the development of real-time measurement tools to better define and replicate human smoking and inhalation behaviours, and to enable equivalent doses to be assessed in laboratory-based biological assays. Re-assessment of previously published data and parallel computational modelling are helping to improve sensitivity analyses to align laboratory findings and dose effects in humans. These tools also help us characterise next-generation heat-not-burn products and e-cigarettes, and are applicable to studies of human inhalation of various substances, such as aerosols produced through combustion, environmental compounds and pharmaceuticals.
Particle size in tobacco smoke and the partition of individual chemical components between particles and the vapour change constantly. Several chemical species have been identified by regulators and scientists as likely key toxicants and potential influencers on disease, due to their observed biological actions in humans. Thus, it is important to identify the doses of individual smoke components and whole smoke that are delivered to specific tissues and organs. We assess the location of deposition, concentration, duration of exposure and mechanisms of removal by chemical reaction or excretion. Our main focus is on the lungs.

Characterisation becomes even more important with novel products, where the aerosols may be quite different from cigarette smoke. To assess these new products, we may need to develop new techniques and models that provide information on how the products are used and where in the respiratory system the aerosols are likely to deposit.

**Recent successes**

A study of regional smoke deposition from multiple combustible products was conducted in a volunteer group, and the results have been used to build and validate broader deposition models in-house and with external collaborators. A pre-study analysis showed that the duration of each step of the smoking process was important, leading to development of a novel time-alignment process for all instruments that derives a unique data set on smoke particle size, concentration, volume, flow and duration of puffing, mouth-hold, inhalation, lung-hold and exhalation. In parallel, we created anatomically correct, optically transparent models of the mouth and upper airways and a puff-inhale-exhale engine to replicate human smoking behaviour in the laboratory and to conduct smoke visualisation studies. The regional deposition studies have generally shown that mouth-hold and mouth deposition are more substantial than previously thought, which has important implications for assessing the sensory attributes of smoke. Deposition patterns in the lungs suggest higher local doses in the upper airways.

Our real-time measurements of smoke aerosol have been widely cited and adopted more broadly within the tobacco research.

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JOHN MCAUGHEY
Principal Scientist

John is an experienced aerosol scientist, having conducted research and published in the fields of tobacco smoke science, particulate air pollution, automotive emissions, pharmaceutical inhalers, radiation dosimetry and occupational medicine. He has previously served on a number of UK Government scientific committees.

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Figure 1: Particle Image Velocimetry image from smoke plume entering mouth cast.
We have adapted these systems for measurement of e-cigarette aerosol and shown excellent precision (<5%) for size and concentration measurements on an individual-puff basis. Further work will be undertaken to agree potential standard testing regimes between industry and regulators.

To improve understanding of regional doses of individual smoke components, particularly in the vapour phase, we have combined physical measurements and modelling. We have supported development of a novel soft ionisation process for time-of-flight mass spectrometry. Measurement precision and accuracy have been validated for seven test chemicals and a proof of concept has been shown for measurement of exhaled smoke.

A review of the literature and vapour-phase computational modelling have enabled us to re-assess partial vapour pressures of compounds and their effects on deposition. Other work has investigated the relative deposition, uptake and reactions of formaldehyde, acetaldehyde and acrolein in the upper airways through hybrid computational fluid dynamic and physiologically based pharmacokinetic models. The data have been compared with previously published non-adverse effect levels and validated. This work has provided a focused complement to the margins of exposure approach used in risk assessment.

Direct measurement of smoke dose in disease assays has been made possible by the integration of a quartz-crystal microbalance in our in vitro whole smoke exposure chamber. Our scientists can accurately and directly determine the mass of smoke in contact with in vitro cell cultures within the nanogram range. Doses of 200–26,000 ng cm⁻² have been quantified over 30 minute exposures, a range similar to that calculated for the daily dose to the upper airways in human smokers. Using this technique, we have calculated that each cell is exposed to between 11 and 1,300 smoke particles per exposure, depending on the smoke concentration. At the lowest concentration, each cell’s exposure represents 400 fg (1 fg equals 10⁻¹⁵g) of smoke particles.

**Aims for the next 2 years**

- Continue to address smoke–particle behaviour with better integration of modelling and measurement components and real-time tools
- Identify and study specific processes that determine initial particle size, concentration and chemical composition, coagulation, condensation, evaporation and hygroscopic growth to improve understanding of particle dynamics and partition in regional deposition
- Extend regional deposition estimates to key toxicants in the vapour phase of inhaled smoke and exhaled breath to improve computational toxicology models
- Apply research to next-generation tobacco and nicotine aerosol products to generate reproducible output on a puff-by-puff basis

**Challenges requiring collaborations**

The continuing scientific challenges are diverse, given the complexity of tobacco smoke, but improving our understanding of the relevant processes will support product stewardship activities for combustible and non-combustible products. We have established close working relationships with leading aerosol modellers in the scientific community, leading to unique and comprehensive data sets. For heat-not burn and e-cigarettes we will build on current aerosol measurements and assessments.

Our investment in real-time measurement and visualisation methods will lead to the development of screening tools and enable objective sensory measures, which will speed the development of products. The tools will provide improved quantitative data on doses to support evidence-driven toxicology models, helping our efforts on alternatives to animal models by developing more advanced toxicological approaches.

Our progress in the measurement field has been particularly assisted by collaborations with the majority of relevant instrument suppliers worldwide.
In vitro tools for assessing disease progression

Toxicity pathway testing using in vitro models is a key feature of innovative toxicological measurements to determine the potential biological impact of products from the cosmetics, pharmaceutical and chemical industries. Adverse outcome pathways (AOPs) have evolved from traditional toxicity pathway testing. We are using the principles of an AOP approach to develop a series of models related to key events associated with the development of cancer, COPD and CVD. The information from such models will contribute to a weight-of-evidence data package to demonstrate the potential of next-generation products to present reduced health risks as compared to conventional tobacco products. In addition, the in vitro models can be used to provide data on the impact of single toxicant exposure, thus increasing our understanding of the contribution that individual toxicants make to toxicity and disease risk.
We firmly believe in the need to develop alternatives to animal testing, both because of the ethical issues surrounding animal testing and that in vitro models of disease-relevant endpoints could provide more appropriate and meaningful information on the biological effects of tobacco-related products. The need for in vitro models is further highlighted by the fact that in vivo animal models do not always accurately reflect human biology. Given that smoking causes a wide range of chronic diseases, including lung cancer, COPD and CVD, we believe that a suite of in vitro models representing key events in disease development need to be established using human tissues. An appropriate suite of in vitro models may allow us to qualitatively assess our current and next-generation products. Additionally, these models can help the rapid and effective screening of new technologies and products, and potentially aid in the discovery and development of new biomarkers of biological effect. Using the AOPs as a framework, we are developing in vitro models to investigate the cellular and tissue responses of smoke during COPD, CVD and lung cancer disease progression. We recognise that in vitro models must be metabolically competent and able to assess chronic damage. Biological processes related to inflammation and oxidative stress underpin more than one smoking-related disease, and so we have also developed in vitro models to better understand how these processes may initiate disease development. Although some of the in vitro models developed are relatively simple, we are also looking at more complex models that could represent processes occurring in the latter stages of disease development. We have invested significant effort in developing a more encompassing approach to standardise our cigarette smoke extracts and to quantify the dose of whole smoke that our in vitro models are exposed to. Use of a quartz crystal microbalance has proved extremely effective in directly determining the mass of whole smoke in our in vitro exposure chamber. Publishing extensively on this new technology is now an important part of making such models robust and relevant.

Recent successes

Over the past few years, in-house studies have focused on the development and application of a suite of in vitro models of disease-relevant endpoints to assess reduced toxicant prototype cigarettes. These prototypes have been tested for CVD and COPD-related endpoints (inflammation and oxidative stress) following exposure to whole smoke, total particulate matter and aqueous cigarette smoke extracts. In relation to CVD, we demonstrated that endothelial repair following exposure to cigarette smoke particulate matter was sensitive to cigarettes with varying toxicant yields. We also determined that osteopontin, an endothelial-specific protein, might contribute to inflammation and subsequent CVD in smokers. With respect to carcinogenesis, several of our publications over the last two years have assessed the utility of measuring DNA double strand breaks using a novel technique of assessing the in vitro genotoxicity of cigarette smoke. We have also been working with a UK clinical research organisation to develop an in vitro model of goblet cell hyperplasia. This model is pathology-specific and brings together several disease processes associated with COPD into a single end point. Other successes have arisen from collaborations with academic and contract research organisations. An in vitro model of COPD has been used to assess the protein content of airway surface liquid following whole smoke exposure. We identified more than 350 proteins in this fluid, many of which are differentially expressed following exposure to smoke, and which comprise the largest set of proteins yet identified in this biomatrix. This type of proteomic approach, which will be published next year, in addition to genomic and metabolomic studies, could lead to the identification of new biomarkers of biological effect that could be used in future clinical studies. Active engagement...
with the scientific and regulatory communities on the utility of in vitro models of disease-relevant end points for product assessment continues to demonstrate the high level of expertise within our laboratories. Our in vitro research have been published in peer-review high-impact journals and many oral and poster presentations have been made at national and international conferences, all of which are shared in the library of www.bat-science.com.

Aims for the next 2 years

» Engage widely on our suite of in vitro models of cancer, COPD and CVD development for product assessment and to help position next-generation products on a risk continuum

» Build on our current models and continue to develop and validate new in vitro models in collaboration with academic institutions and contract research organisations

Challenges requiring collaboration

The challenges we face in the development and acceptance of in vitro models of disease by the scientific and regulatory communities are best met through collaboration with academic partners, suppliers of testing equipment, contract research organisations, regulators, and others in the regulated industry. In vitro models need to be validated across a range of laboratories for robustness and reproducibility. Continued academic and industrial collaboration is essential to ensure we continue to actively participate in and understand the science that surrounds tobacco-related disease and the development of appropriate in vitro models. Much of the in vitro modelling work in the pharmaceutical and chemical industries overlap with our research, and we think it important to broaden collaboration across a wide group seeking to reduce and eventually replace animal experiments. The guiding principles for the development of in vitro models remain applicable to the work in GR&D. We hope that developing active engagement with the US FDA and other regulators will continue to ensure that our research is of relevance and of the highest quality. Finally, NIH/US FDA funding for our academic partners through joint academic–BAT applications will also be pursued, demonstrating our intent to participate in high-impact research with all our external collaborators.

Figure 1: A diagram to illustrate our approach using AOPs as a framework to develop in vitro models of disease-related endpoints. We are developing several in vitro models of relevant events in the development of three major tobacco-related diseases, COPD, CVD and lung cancer, and their underlying processes.
Although much can be gained from chemical, physical and toxicological tests in the laboratory, studies of the way people use products and the biological consequences of such use are essential to the assessment of a product’s potential to reduce health risks as compared to cigarette smoking. We are exploring a wide range of techniques to assess both toxicant exposure and the potential biological consequences of these exposures, and we have undertaken several studies that illustrate the potential methodological difficulties involved in long-term clinical studies of these effects.
Laboratory assessment of how novel products perform is an essential part of product development, and a comprehensive battery of chemical, physical and biological tests is required. These data, however, cannot fully predict how consumers will use products or what the short- and long-term effects of product use might be.

In early 2012, the US FDA presented its priorities for tobacco research as 56 questions on a wide range of topics, including the diversity of tobacco products, addiction, toxicity, carcinogenicity and the adverse health consequences of tobacco use. We had already undertaken human volunteer studies to address some of these questions, ranging from subjective sensory assessment to objective tests of use behaviour such as puffing and inhalation measures. Methods to estimate exposure (or dose) to toxicants and other components in combustible and oral tobacco products were also developed. This testing provides useful information about mouth-level exposure to nicotine and tar from tobacco products, but cannot demonstrate toxicant uptake into the body or biological effects, for example after switching from one product to another.

Since 2005, we have been conducting biomarker studies in clinical environments. We measure biomarkers of exposure — smoke constituents or their metabolites — in biological samples (e.g. urine, blood or saliva) to assess uptake by the body and the duration of exposure. Biomarkers of biological effect are chemicals or other markers produced by the body in response to these exposures, and can indicate immediate consequences of product use. We assess changes with the aim of identifying their roles in the processes that could lead to smoking-related diseases.

Our clinical studies typically involve volunteers who visit clinics (on a residential or non-residential basis) to ensure complete sample collection, assess compliance, control for confounders (e.g. diet and environmental sources), enable assessment of end points that require specialist equipment and ensure the safety of participants through medical observation and testing. Similarly to other industries involved in clinical research, we prepare and publish detailed protocols, register trials on internet sites before they begin, obtain written informed consent from all participants and ethics approval from independent ethics committees, and adhere to principles of good clinical practice.

Recent successes

We have completed two clinical studies where smokers in test conditions were switched to cigarettes with reduced yields of certain smoke toxicants. The first study concentrated on toxicant exposure (assessed by measurement of biomarkers of exposure) in smokers who switched from conventional cigarettes to a reduced toxicant prototype cigarette for four weeks. The findings showed a good correlation between reduced yields of certain toxicants (as measured on smoking machines) with corresponding biomarkers of exposure. In fact, some biomarker levels, particularly those related to some vapour phase toxicants, were reduced by up to 70–80%.

The second study involved switching volunteer smokers to a reduced toxicant prototype for 6 months, with effects as assessed by measurement of biomarkers of exposure and biomarkers of biological effect. The study protocol was published earlier this year and we expect to publish

![Biomarkers of exposure](image_url)

**Figure 1: Example reductions in toxicant biomarkers for an RTP1 prototype.** For more detailed results see: DOI 10.1016/j.yrtph.2013.02.007
CLINICAL STUDIES

Figure 2: Diagram outlining 6 month clinical study on a second generation of RTPs, see clinical registration number ISRCTN81286286.

the clinical findings from this study soon. Initial results on behavioural changes have already been presented to a meeting of the US FDA's Tobacco Products Scientific Advisory Committee. One of the key limitations of such studies is the lack of validated biomarkers of biological effect relevant to smoking-related diseases. In this study, we also measured potential biomarkers of biological effect in ex-smokers and people who have never smoked, which may help to identify potential biomarkers of biological effect for future studies.

A longitudinal (3.5 years) observational study has also been completed, and this sought to investigate the effects of spontaneous switching between commercial brands of cigarettes with different ISO tar yield and to estimate mouth level exposures to nicotine and tar, with measurement of biomarkers of exposure every 6 months. This study greatly improved our understanding of the variability in smokers' exposure over time. The study is expected to help in the design of post-marketing observational studies of MRTPs.

We have also published the results of a nicotine pharmacokinetic study that compared the kinetics of nicotine uptake from various forms of pouched and loose snus with that from cigarettes and an over-the-counter nicotine gum. After a single use of each product, multiple blood samples were taken over a 2-hour period. Nicotine was absorbed more rapidly from the cigarette smoke than snus, but the total systemic exposure was similar overall.

Aims for the next 2 years

» Analyse and publish the data from our long-term clinical study
» Continue to seek, though collaborations, biomarkers of biological effect that could be meaningfully used in studies with potentially reduced-risk products
» Develop new techniques and undertake studies that give insights into consumer use of next-generation tobacco and nicotine products

Challenges requiring collaboration

There remain substantial challenges in conducting clinical studies to assess potential reductions in risks associated with new products, and these would best be tackled through collaboration. In addition, assessing new products to population health standards (that is, determining the potential impact on non-tobacco users as well as tobacco consumers) will require new tools for modelling population effects. An effective statistical model capable of predicting the impact of the marketing of a modified risk tobacco product (MRTP) on the overall risk to the population would be a powerful tool that would benefit all researchers in the field, and would be subjected to continuous improvement as more data was applied to the model. We believe that population models that predict the impact of regulatory and public health actions on tobacco use should form the base model, with the potential impact of novel products built as a factor that could influence population tobacco and nicotine use, and consequently population health outcomes.
Good research practice

There are numerous reasons why it is important for research establishments, and particularly those that live in an environment that, unlike pharmaceuticals, is not as yet tightly prescribed by regulation, to develop and live good research practice. Doing so improves the integrity of the research, ensures that the research can stand up to scrutiny in both peer-review papers and in applications for intellectual property rights, and improves the processes of research to make them more efficient and effective.
Science-based research environments typically incorporate innovation, discipline and a passion to question, explore and create. To conduct research consistently and maximise its benefits can be challenging, especially in large, and frequently complex, organisations. Good research practice underpins the integrity, quality and validity of high-quality science and increases confidence and trust in the research process. Thus, the creation of frameworks that provide a clear understanding of scientists’ responsibilities to the company and wider society is important. We are embedding principles, expectations, accountability and responsibility into the way our scientists work.

To formalise these principles and expectations, a code of conduct has been created that has its origin in the practices of the wider scientific community. This is essential as we undertake research to reduce the health risks of our products in existing and new categories so they will meet the criteria of regulators and consumers. The code outlines the expected behaviours of scientists and minimum standards for how scientific research work is conducted, reported and applied.

Experiments should be conducted on the principles of established science with the goal of establishing new knowledge. Standards, use, calibration and maintenance of premises and equipment are essential to good practice and robust results. Reagents and reference standards should be carefully selected, and guidelines/controls put in place for hazardous materials/activities. Collection, storage and retention of primary data and samples should be carried out according to established practices. Finally, documentation, recording and reporting of results should be comprehensive and clear.

**Recent successes**

Honesty and transparency are instilled in all of our research, in respect to scientists’ own actions and in their responses to those of other scientists. This applies to the whole range of our scientific research activities, including experimental design, generation and analysis of data, publishing of results, and acknowledgement of the direct and indirect contribution of colleagues, collaborators and others. Plagiarism, misrepresentation, fabrication or falsification of results is not tolerated.

We are committed to excellence and set high standards related to research ethics. All employees and third parties engaged in our research programmes that are financed directly by the group, or are done in partnership, are expected to recognise and accept personal responsibility for the integrity of their research. These standards ensure that research findings are robust and defensible.

Although recognising the need to protect intellectual property and commercial interests, we expect scientists to be as open as possible in discussing their work with other scientists and with the external scientific community. This approach helps GR&D scientists to maintain a critical attitude towards their own and other scientists’ findings, which enables freedom of scientific thought and exchange of opinions and information.

All our clinical studies are designed to respect and safeguard the dignity, rights, safety and well-being of the participants. We are required to obtain written informed consent from all participants and we adhere to the ethical principles of the Declaration of Helsinki. Studies are approved by an appropriate external ethics committee, and principal investigators and supervisors are given responsibility to make sure that the procedures in the approved protocol are adhered to. Clinical studies are registered on public databases. Animal studies are not part of our routine testing and are kept to a minimum. We constantly review whether alternative methods are available, with the long-term aim of being able to phase out animal studies altogether.

All research involving genetically modified tobacco plants or seeds have to be approved through our Biological Safety Committee and meet the legal and regulatory standards in the relevant country or countries.

GR&D scientists are expected to apply, where available, the standards of research practice published by scientific and learned societies and other relevant
professional bodies. Scientists are also expected to be aware and keep up to date with the legal requirements relevant to their scientific field of research.

**Aims for the next 2 years**

- Publish on the ethics of tobacco research
- Continuously improve our approaches

**Challenges requiring collaboration**

Collaboration in research can lead to best practices across a range of processes that improve the way in which research is performed. This can include planning and design of experiments, and the development of strategies to avoid negligence, haste, carelessness and errors.

All of our work is reported formally, e.g. internal reports, publication in peer-review journals and presentations at meetings/conferences while taking into account intellectual property and commercial opportunities.

We think that the process of peer-review publications and presentations at conferences, and the way in which we seek input to our programmes before they are finalised, improves the quality of our research. Collaboration with the scientific community is essential to this.

Training and development is a key component of good research practice. All GR&D scientists must undertake relevant and appropriate training, for example in research design, statistics, regulatory and ethics approvals and consents, methods and equipment use, confidentiality, data management, record keeping and data protection. We also strongly recommend that GR&D scientists who belong to professional bodies participate in continuous development plans to keep their skills and capabilities current.

For good research practices to work, the responsibility for promoting and delivering robust results is shared by all those involved in generating scientific evidence for the business, our consumers and regulators. As an organisation we encourage and nurture a culture that supports and embeds its principles and aims to prevent research misconduct.

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**Figure 1: Fundamentals of Good Research Practice at British American Tobacco.**
Electronic cigarettes

E-cigarettes are a relatively new category of product, but both the large amount of interest from cigarette smokers and the support from some regulators and their advisors for them as a potential substitute for cigarettes is driving rapid growth in the number of related scientific publications. It is the toxicants in tobacco smoke, not the nicotine, that causes most smoking-related risk; however, e-cigarettes may not be risk-free and scientific analysis is needed to more fully characterise them and their use.
We are committed to tobacco harm reduction and believe that e-cigarettes have the potential to play an important role in this. If manufactured to appropriate quality standards, e-cigarettes might represent a significant step forward for many smokers who are unwilling or unable to stop smoking conventional cigarettes. Currently, they are banned in some countries, regulated as medicinal products or as consumer products in others. BAT has committed to commercialise regulated nicotine products, including e-cigarettes, through Nicoventures.

The UK Royal College of Physicians, following the thoughts of Dr Michael Russell in the 1970’s, has concluded that although nicotine is addictive, it is the toxicants in tobacco, and particularly in tobacco smoke, that do the vast majority of the damage caused by smoking. Although nicotine can have cardiovascular effects, and there are concerns about its use during pregnancy, there is very little evidence of adverse effects from long-term use; thus, some authorities consider medicinal nicotine to be relatively ‘safe’. Recently, two researchers in this field, McNeill and Munafò, have suggested that there is a continuum of risk from the most dangerous, combustible tobacco products such as cigarettes, cigars and pipes, to the least dangerous non-combustible nicotine products such as nicotine replacement therapy (NRT) and e-cigarettes.

E-cigarettes are battery-powered devices that produce an aerosol usually containing nicotine. Disposable e-cigarettes are typically cigarette-shaped and sized whereas reusable devices are frequently larger and more complex.

When a consumer draws air through the device, a heating coil at about 150°C wound around a fibre glass or ceramic core vaporises a liquid formulation (or ‘e-liquid’) drawn onto the coil by capillary action. A proportion of the vapour produced condenses to form an aerosol that can be inhaled. The characteristics of the aerosol formed depend mainly on the power applied to the heating coil, the physical characteristics of the formulation (e.g. viscosity and wettability) and the specific heat capacity of the formulation. The liquid formulation usually contains excipient (glycerol, propylene glycol and/or water), nicotine and flavourings. Typically, a coloured LED indicates when the device is active.

E-cigarettes are relatively new and there are scientific questions to be answered, including what standardised regimes should be used for their chemical and biological characterisation. We are beginning a research programme to study the key issues surrounding e-cigarettes.

Recent progress

Literature reviews suggest that the extent of degradation of glycerol and propylene glycol (common excipients in e-cigarettes) at e-cigarette temperatures is unclear, as are the formation characteristics of potential degradation products (particularly formaldehyde, acetaldehyde, acetone and acrolein). Initial GR&D experiments to characterise this degradation have been undertaken using thermogravimetric and infra-red analyses to study the compounds evolved during heating, but further e-cigarette-specific experiments are planned.

Particle measurements have been made on a range of e-cigarettes. The aerosol produced by a commercially available e-cigarette with a formulation of glycerol, water and nicotine had similar particle sizes to tobacco smoke, as measured by electrical mobility, laser diffraction, and similar particle concentration. These attributes may be particularly important to provide sensory characteristics that are similar to tobacco smoke and achieve nicotine uptake and pharmacokinetics sufficient to encourage cigarette smokers to substitute.

Standardised regimes for machine testing of e-cigarettes are important for comparison of products and characterising chemical and physical composition of the aerosol. E-cigarettes have been ‘smoked’ on cigarette smoking machines using a specific regime that takes into account both existing knowledge of consumer behaviour and the performance of the devices. Testing has shown that the aerosol composition can be...
consistent with that of the e-liquid, with no toxicants above detection limits, but can also, depending on the formulation/device, contain measurable amounts of impurities, such as nitrosamines, and thermal decomposition products, such as the aldehydes mentioned previously.

There are very limited data available describing the consumption patterns of e-cigarette use, and although assumptions can be made to guide the product development and assessment processes, a comprehensive study of consumption behaviour will be made. In addition, a detailed study of puffing and inhalation behaviour will be undertaken. This will inform biological (in vitro), chemical and physical testing and enable an assessment of pulmonary deposition/retention to be made.

**Opportunities for collaboration**
Given that e-cigarettes are a relatively new product category, there are many opportunities for researchers to collaborate in the developing science. In particular, the development of common testing techniques and analytical methods for analysis is best done in collaborative partnerships. Unlike many product categories, e-cigarettes are extremely diverse in their design and performance, and researchers could be assisted also by the development of some standardised reference products.