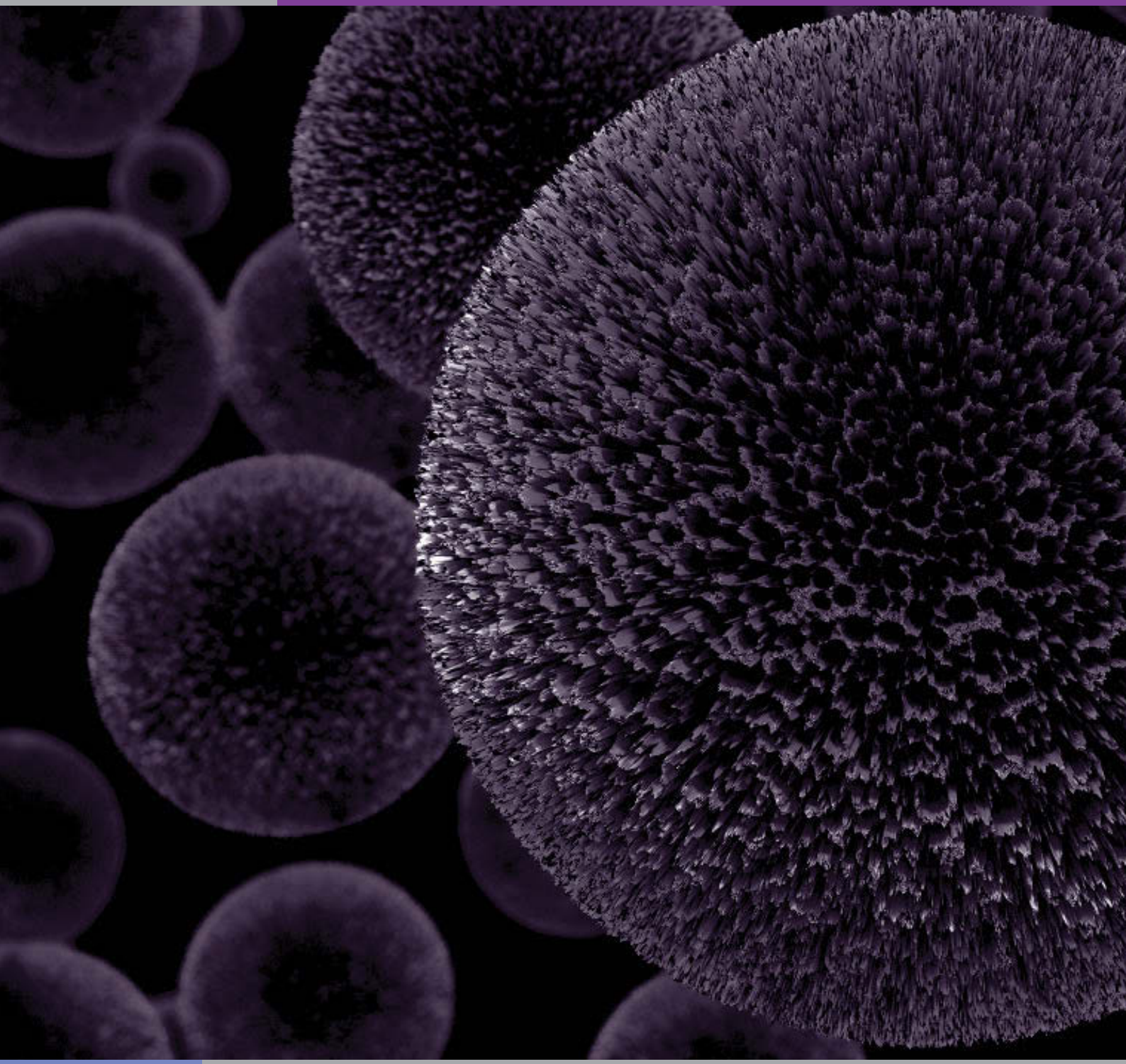
reduced toxicant prototypes

reducing smokers' exposure to cigarette smoke toxicants



inside: scientific challenges | tobacco toxicants | building a prototype | testing

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British American Tobacco

Cover Image: Artist's impression of a nanoporous carbon used in the filters of reduced toxicant cigarettes. A huge internal surface area allows for very effective trapping of certain smoke toxicants deep in the carbon pores.

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About this Report

This is a British American Tobacco p.l.c. report describing some of our research towards developing a potentially reduced toxicant product. It reports on some of the activity of British American Tobacco Group Research & Development in the UK. References to 'British American Tobacco', 'we', 'us' and 'our' when denoting opinion or activity refer to British American Tobacco Group Research & Development.

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British American Tobacco

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Can cigarettes ever be made safer?

Dr David O'Reilly
Group Scientific Director

David directs British American Tobacco's scientific research, which seeks to better understand the underlying mechanisms of smoking-related diseases and the development of reduced toxicant products. He has been a driving force in the expansion of British American Tobacco's R&D into an external-facing, multidisciplinary environment with hundreds of scientists working towards reducing the health impact of our products.

We don't honestly know for certain, but cutting edge science is our best chance. We are under no illusions as to the complexity of the problem. As we like to say: when it comes to tobacco science, it's not rocket science, it's harder!

People have used tobacco for thousands of years, although the cigarette did not come to prominence until the last century. Prior to that, tobacco was used mainly as snuff or cigars, the smoke of which is not normally inhaled. By contrast, tobacco in cigarettes is burned and inhaled deeply. It is really only in the last 50 years that the serious impact of cigarette smoking on health has become apparent.

Nobody seriously disputes that tobacco causes serious and fatal diseases like lung cancer, chronic obstructive pulmonary disease, and cardiovascular disease. And our scientists and others have been working to understand what it is about cigarette smoke that produces these serious health problems.

Tobacco is a plant and burning it, like burning any plant material, converts thousands of plant-based compounds into thousands of other compounds, some of which have been identified as toxic.

Many have concluded that exposure to these toxicants, whether individually or as interacting mixtures, is the root cause of smoking-related diseases. This leads naturally to several important questions: which of these toxicants are the most relevant?; can their presence be reduced or eliminated?; and will this have any impact on smokers health?

Identifying the toxicants most likely responsible for the development of smoking-related diseases is very difficult, primarily because we are still discovering how these toxicants interact with the body to cause disease.

> When it comes to tobacco science, it's not rocket science, it's harder!

Once the important toxicants have been identified, the next step is to develop technologies that when incorporated into cigarettes, provide meaningful reductions in toxicants levels in the smoke.

And finally we must determine if reducing or eliminating any of these toxicants has any effect on smokers' exposure levels and if so whether this has any effect on health. This is the hardest part. The nature of tobacco-related diseases is such that they can take decades to manifest themselves, which means that today, a real scientific challenge is to develop testing regimes that reliably predict future disease outcomes. Reducing the toxicants in cigarette smoke may eventually be shown to reduce a small portion of the health risks. However, substantial health risk reduction will need the development and evaluation of a new generation of products, including smokeless and pure nicotine products.

The origin of toxicants in smoke

It is well established that the risks of smoking are greater in people who smoke more cigarettes per day and for longer periods[1]. It therefore makes sense that inventing cigarettes with substantially less toxicants in the smoke might reduce some of the health risks. But first we must understand where these toxicants come from and which are the most important in terms of health.

Dr Kevin McAdam
Senior Principal Scientist

To date, scientists have identified thousands of different chemical species[2] in the smoke of a burning cigarette, and there are indications that there may be tens of thousands more. Of those already identified, more than 150 are believed to be toxic. These smoke toxicants are formed either by the evaporation of tobacco constituents, the thermal fracturing and decomposition of molecules in the tobacco, or chemical reactions occurring in the hot gases generated by the burning tobacco.

The main component of a cigarette is, of course, tobacco. Tobacco is a member of the *Solanaceae* family of plants; a wide-ranging group that includes potatoes, tomatoes and chilli peppers. Like all plants, tobacco is a chemically diverse biological material made up of large molecules such as cellulose, starch, pectin, and proteins, as well as a broad range (again, several thousand) of other chemical compounds.

The exact chemical composition of tobacco, and therefore the toxicants produced when it is burned, is strongly influenced by many external factors, including the type of soil it is grown in, the way it is grown (agricultural practices), the position of the leaf on the tobacco plant and the curing (drying) process post-harvest (Figure 1).

Chemical Composition of Smoke

The growing tobacco plant takes up nutrient minerals from the soil and transports them to the leaves. As a consequence of this transport process, toxic heavy metals such as

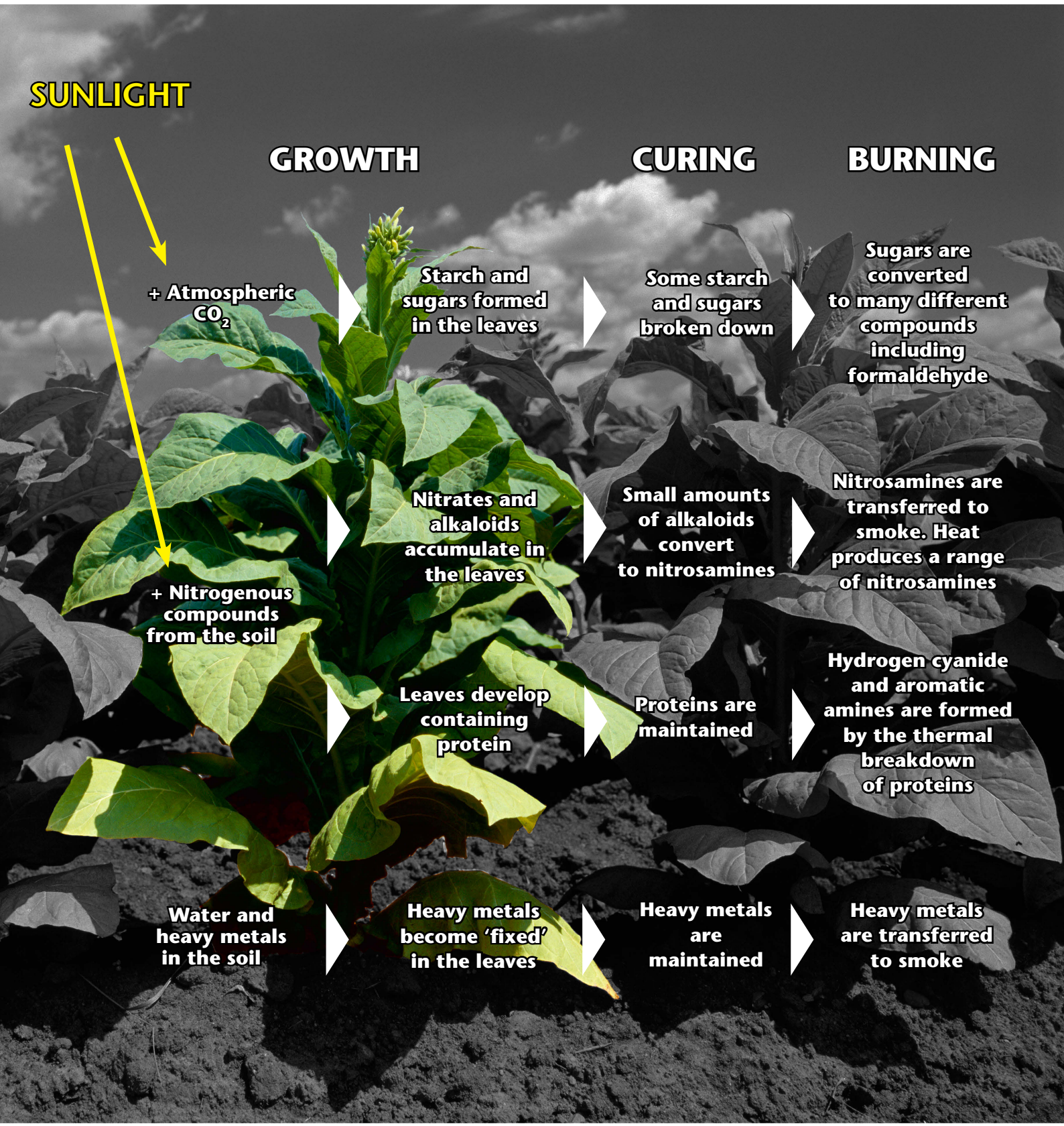


Figure 1. Routes to toxicant formation.

arsenic, cadmium and lead accumulate in the leaves. These metals are then transferred to tobacco smoke when the cigarette is burned.

Some toxicants are also formed during the curing process, when the green tobacco leaves dry and turn yellow-brown. For example, at this stage a portion of tobacco alkaloids undergo chemical changes producing compounds called tobacco-specific nitrosamines, some of which are carcinogenic.

When the tobacco is burned, toxicants accumulated in the leaf during growing (for example, heavy metals) and toxicants produced during the curing process are transferred to the smoke. Additional toxicants are formed as a result of burning.

As the tobacco burns, some of its more thermally mobile constituents, such as tobacco-specific nitrosamines, evaporate (or 'distill') into the smoke in

a similar way as water boils from a kettle. Other tobacco constituents, such as metals or nicotine, are chemically bound within the tobacco but released into smoke by the heat of the tobacco coal.

However, some tobacco constituents such as sugars, cellulose and proteins are too immobile or involatile to evaporate. The heat causes them to break down into a diverse range of smaller, simpler and more volatile chemical constituents. These include water, carbon dioxide and carbon monoxide, as well as many thousands of other combustion products. Tobacco sugars, for example, thermally degrade to a range of compounds including formaldehyde, a respiratory carcinogen. Cellulose degrades to carbon monoxide and a wide range of compounds, including the known carcinogen benzo[a]pyrene.

Formation of Smoke

When a cigarette is lit and the smoker takes a puff, the temperature at the lit end can reach over 900°C — four times hotter than a conventional kitchen oven. The intense heat causes the tobacco to char and carbonize, producing smoke.

Cigarette smoke starts out as a highly-concentrated vapour behind the burning coal. As it travels down the tobacco rod towards the mouth, the rapid decline in temperature, together with nucleating sites within the gas, causes condensation of the vapour into aerosol particles of varying sizes.

This dense cloud of aerosol particles is drawn down the cigarette towards the cigarette filter by the puffing action of the smoker. As the smoke travels down the tobacco rod the smaller particles can grow in size due to coagulation and condensation; some of the particles are removed from the smoke stream by impaction onto the tobacco and filter fibre surfaces. Most of today's cigarette filters are composed of cellulose acetate fibres; these mechanically filter smoke particles from the smoke stream and reduce the total weight and number of aerosol particles in the smoke. When travelling through the filter, the smoke stream can be mixed with diluting air coming into the cigarette through ventilation holes in the cigarette filter. Some cigarette filters also contain active carbon particles, which remove a portion of the volatile constituents of smoke. The smoke exiting the cigarette filter during a puff is inhaled by the smoker.

A modern cigarette contains just over half a gram of tobacco. From each cigarette, smokers generally inhale about a hundredth

Smoke constituent	Sources
Tar	All of the material in the cigarette – a combination of combustion products and distilled compounds
Nicotine	Tobacco leaf
Carbon Monoxide	All of the carbonaceous material in the tobacco leaf
Polynuclear aromatic hydrocarbons (PAHs), including benzo[a]pyrene	Tobacco phytosterols, aliphatic hydrocarbons, long-chained terpenoids, PAHs on leaf
Tobacco-specific nitrosamines (TSNAs), including NNK* and NNN** * 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone ** N'-nitrosonornicotine	TSNAs in the tobacco; pyrosynthetic reactions of tobacco alkaloids as the tobacco burns
Aromatic amines, including 1-aminonaphthalene, o-toluidine and 3-aminobiphenyl	Tobacco proteins and amino acids
Formaldehyde	Tobacco polysaccharides such as cellulose, starch, pectins, lignin, natural and added sugars, and glycerol
Acetaldehyde	Tobacco polysaccharides such as cellulose, starch, pectins, lignin, natural and added sugars and glycerol
Acrolein	Tobacco polysaccharides such as cellulose, starch, pectins, lignin, natural and added sugars, and glycerol
Crotonaldehyde	Tobacco amino acids, waxes and complex sugars
Benzene	Tobacco amino acids, waxes, complex sugars
Hydrogen cyanide (HCN)	Tobacco proteins, amino acids
Metals such as arsenic, cadmium, chromium, lead, mercury and nickel	Metal compounds within tobacco (e.g. arsenic oxides present in the leaf)

Nicotine

The best-known compound in tobacco is the alkaloid nicotine. It is also found in other members of the *Solonaceae* family such as tomatoes, but to a lesser extent. Nicotine is physiologically active and generally present at 0.5–3% of tobacco by weight. At the low doses synonymous with tobacco and cigarette product use, it acts both as a stimulant and a relaxant, a property known as a 'bi-phasic physiological response'. Nicotine is not the direct cause of tobacco-related illness, although it may have an effect on the cardiovascular system.

Tar

The majority of smoke is composed of gases such as nitrogen and carbon dioxide. But mixed with these gases are multitudes of other gaseous and vapour compounds that are in evaporative equilibrium with billions of small aerosol particles. These particles scatter light, causing the white appearance of cigarette smoke. When analysed the aerosol particles (except for the nicotine and water content) are measured collectively as the gravimetric quantity 'tar', which is the collective weight of the liquid and solid products of burning tobacco; it contains a high proportion of the thousands of smoke constituents and is a product of combustion rather than an additive to cigarettes.

of a gram of tar, a thousandth of a gram of nicotine, a few millionths of a gram of formaldehyde and a few billionths of a gram of benzo[a]pyrene and lead. It is the cumulative effect of many years of exposure to very small quantities of smoke toxicants that leads to the onset of smoking-related diseases.

Toxicological Prioritisation

The risks of smoking are greater in people who smoke more cigarettes per day and for longer periods, so it has been suggested that inventing cigarettes with substantially lower toxicants in the smoke might reduce some of the health risks. Understanding which smoke toxicants are associated with smoking-related diseases (and their relative contributions) is therefore an obvious first step towards reducing the harm associated with smoking. To date, more than 150 constituents have been identified as potentially toxic. The challenge facing harm reduction scientists is to establish which of these should be prioritised for reduction or removal.

A number of scientists have attempted to prioritise key toxicants and compile lists of toxic smoke constituents that may be important in smoking-related diseases. However, the lists are not in complete agreement, and on a quantitative population basis these models fail to predict the observed incidence of smoking-related diseases. Thus, a reliably prioritized list of smoke constituents that accounts for the health hazards of cigarette smoking has yet to be developed. More sophisticated approaches are now being developed to do just that: scientists are, for example, looking at how mixtures of toxicants behave, rather than just considering each toxicant in isolation. In addition, physiological models that more accurately reflect what is happening in the body are being developed, as well as *in vitro* biological test systems to evaluate toxicant behaviour in the lab. Together, these recent approaches seek to assess the probability of human uptake of a dose sufficient to provoke adverse cellular change during normal smoking behaviour.

In Europe, existing cigarette regulation strategies focus on machine measurements and limits on tar, nicotine and carbon monoxide emissions. Other countries, such as Brazil, Canada, and Taiwan expect manufacturers to report annually on the machine emissions of a number (up to 44) of toxicants in cigarette smoke. In the US, the Food and Drug Administration (FDA) has recently established a list of Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke[3].

The FDA also has the authority to establish standards, including provisions for the reduction in emissions of smoke constituents. A World Health Organization study group (TobReg) recently proposed mandated lowering of toxicant yields from cigarettes as part of a product regulatory strategy[4]. The toxicants selected were based on considerations such as animal and human toxicity data, hazard indices and the potential for the toxicant to be lowered.

Conclusions

Achieving reductions in emissions of smoke toxicants will require a thorough understanding of tobacco composition and how cigarettes burn, as well as validated analytical measurement methods. Changes to tobacco composition, the introduction of new cigarette papers and the use of filter additives are all valid approaches to reducing cigarette smoke emissions. However, whether these reductions will reduce health effects of cigarette smoking will require substantial levels of proof, including clinical data on changes in toxicant exposure during real-world use, evidence that these reductions are biologically meaningful, and an appreciation of the impact of these changes on smoking populations (see pages 10-12).

1. Doll *et al.*, Mortality in relation to smoking: 40 years' observations on male British doctors. *BMJ* 1994; **309**, 901–911.
2. Rodgman, A. & Perfetti, T. A. The complexity of tobacco and tobacco smoke, *Beitr. Tabakforsch. Int.* 2011: **24,** 215–232
3. US Food and Drug Administration [Docket No. FDA-2012-N-0143] Harmful and potentially harmful constituents in tobacco products.
4. Burns D. M. *et al*, Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organisation TobReg proposal. *Tob Control* 2008; **17:** 132–141.

Building a prototype reduced toxicant product

Our search for toxicant-reducing technologies means that we have been investigating each element in the construction of a cigarette — including the tobacco blend, the cigarette paper and the filter — looking for opportunities to modify toxicant levels in smoke, either by restricting their formation or reducing the amounts once formed. Technologies capable of reducing levels of toxicants in tobacco smoke must be compatible for use in a cigarette such that it is still acceptable to consumers and in a way that does not inadvertently affect the levels of other smoke toxicants.

Dr James Murphy
Reduced Toxicant Prototype (RTP) Project Manager

Cigarette smoke is a very complex dynamic mixture containing thousands of reacting and interacting compounds; many of these compounds have already been identified, but many thousands more have not. Some have been classed as toxicants, and our approach to research aimed at reducing the harm they cause is to reduce the levels of certain smoke toxicants by modifying the cigarette. It is imperative, however, that by altering the complex system that is cigarette smoke to reduce the level of one toxicant, we do not inadvertently cause an increase or promote the formation of others.

Every year, we screen hundreds of candidate technologies for their ability to reduce toxicants in smoke. To be a serious contender, a candidate technology has to significantly reduce toxicant levels, be suitable for use in cigarette manufacture, be acceptable to the consumer and be suitable for scale-up.

There are a number of issues that commonly arise when new candidate materials are being considered. Some materials are simply rendered ineffective by the smoke; and because the materials added to the tobacco roll are burned in the cigarette, there is also the potential for the generation of new potentially toxic compounds .

In terms of filtration, two issues that commonly arise when we investigate new filter additives or materials. First, cigarette smoke is highly complex, and candidate

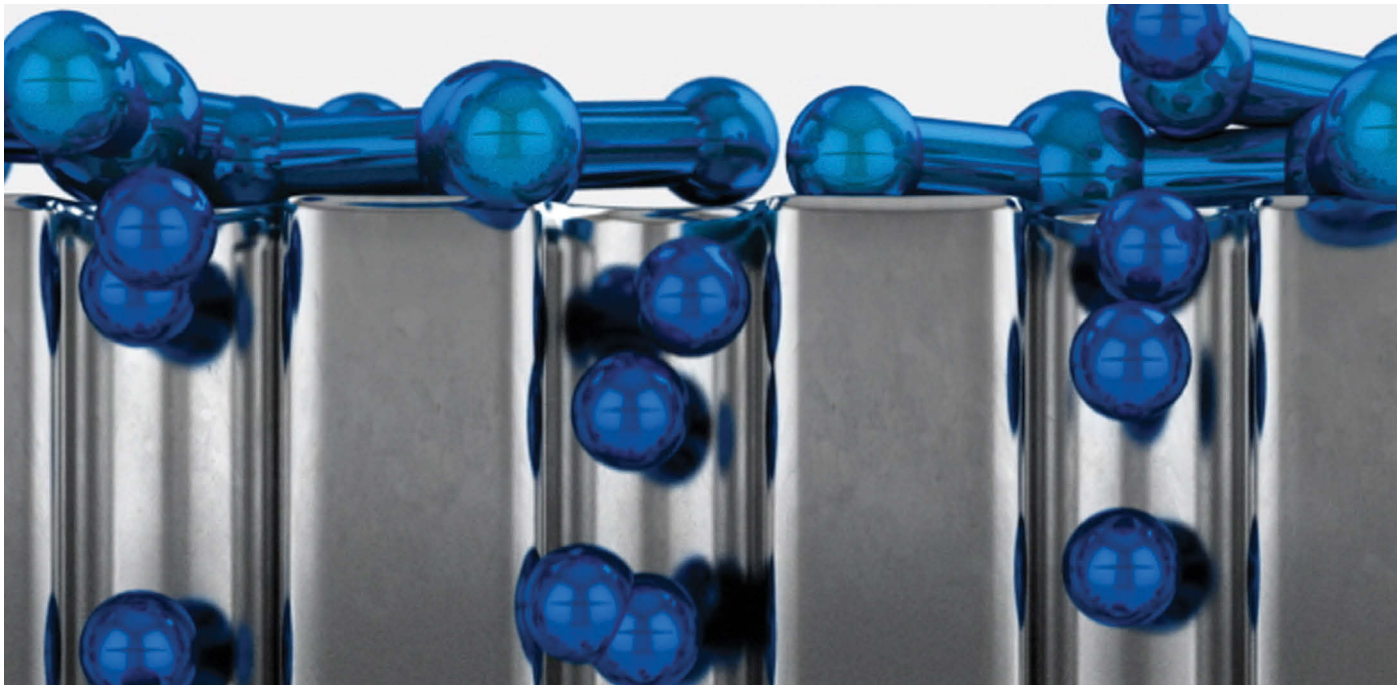


Figure 1. Treating tobacco: after the tobacco is washed, the liquid is passed through a resin that removes certain toxicant precursors in a manner similar to a water filter.

materials are often poisoned or coated by tar in the first puff, rendering them ineffective. Second, residence time in filter is typically very short (<0.01 secs) and as such, the cigarette smoke has passed through the filter before the new material/technology has an opportunity to impact the smoke.

Another barrier to the development of technologies is the ability to incorporate them into a cigarette and deliver an acceptable smoke. In some cases, it is possible to design the blend and cigarette to offset a small reduction in sensory performance, but in others this is not possible without reducing the efficacy of the technology.

Scale-up brings its own challenges, and may be the longest stage in developing a new technology to meet the needs of high speed/volume production.

We have recently developed four technologies that have been incorporated into a series of prototypes that we hope will have reduced levels of toxicants in the smoke. These technologies include a treated tobacco that is, among other things, washed with an enzyme; a glycerol-containing tobacco substitute; a novel nano-porous carbon that is more effective than any carbon currently used in cigarette filters, and an ion-exchange resin similar in concept to those used in water filtration.

Building a Prototype

We have applied two technologies to the tobacco rod and two to the filter. Theses technologies were combined in different ways to produce three different prototypes. Tests in the lab using smoking machines showed that all three prototypes yielded substantially reduced levels of many volatile toxicants compared with conventional cigarettes. However, clinical studies are required to determine if these reductions result in reduced exposure to the smoker (see pages 10-12), and further even longer-term clinical studies will be required to determine if that means anything in terms of health.

Tobacco Technologies

We have used two tobacco technologies: a treated tobacco[1] and a glycerol-containing tobacco substitute[2].

Treating the tobacco involves putting it through a multi-step process using a biological enzyme, a clay powder used in wine production and a food additive. The enzyme breaks down proteins in the leaf that become toxicants when burned, and the other materials act as filters to remove the degraded protein (see Figure 1). The result is a tobacco with substantially less protein and polyphenols (both of which occur naturally in leaf).

The tobacco is initially washed to remove soluble proteins and polyphenols. The washings are then passed through two filters. First, a Bentonite filter removes proteins (this filter is also used in wine production to remove proteins that make the wine cloudy). Second, a polyvinylpyrrolidone filter, also used as a food additive, removes the polyphenols.

In a parallel process, the washed tobacco fibre is treated with a proteolytic enzyme to break down insoluble proteins in the tobacco leaf into smaller peptides that can be washed away.

The liquid and tobacco extracts are then recombined and dried. The enzyme is then deactivated by heat treatments. The dried processed tobacco is then suitable for use in cigarette manufacture.

Tests in the laboratory revealed that the smoke produced by burning the treated tobacco has less of most aromatic amines, hydrogen cyanide and phenols. Of the 43 toxicants tested, reductions were observed in 23. There were, however, increased levels of formaldehyde and isoprene.

The second technology is a tobacco substitute sheet composed of chalk, alginate (a binding agent extracted from sea-weed) and glycerol. It is used as a substitute (typically 20%) for tobacco in the tobacco rod.

The concept of a tobacco substitute sheet is not new. Previous versions used non-tobacco sources of combustible material. We extended this approach in two main ways. First, we replaced as much organic material in the sheet as possible while allowing cigarettes to burn uniformly to produce ash that behaves like a conventional cigarette – that is, ash that does not fall off or blow away, but comes away with a tap. We achieved this using calcium carbonate (chalk) and alginate as a binder. Chalk is inorganic and does not create toxicants when burned. The second development was a substantial increase in the amount of glycerol used in the sheet.

The substitute reduces toxicant levels in two ways: first, because some of the tobacco is replaced with this sheet, there is simply less tobacco available to produce

toxicants; second, when the glycerol in the sheet is burned, it vaporizes becoming part of the smoke, diluting the toxicants.

Tests of cigarettes containing up to 60% of the substitute sheet using smoking machines showed a general reduction in nearly all smoke toxicants measured. Levels of benzo[a]pyrene were reduced by about 20%, heterocyclic amines by 20–50%, tobacco-specific nitrosamines by 20–30% and phenols/cresols by 30–75%[2].

There were, however, increases in some volatile components such as formaldehyde, although levels can be reduced with charcoal filters.

Filter Technologies

There are also two filter technologies — a novel nano-porous carbon[3] that is more efficient than any carbon currently used in cigarette filters, and an ion-exchange resin[4] like those found in water filters.

Standard cigarette filters are made from cellulose acetate, a cotton-like material derived from wood cellulose. Carbon is sometimes added to improve the filter's ability to reduce certain vapour-phase or volatile compounds. This carbon or charcoal is commonly derived from coconut shells.

The carbon used in the prototypes is synthetic, with a novel nanostructure and a huge internal surface area effective for trapping volatile smoke toxicants. The amount of this carbon used in one cigarette filter has an external volume equivalent to a drop of water but an internal surface area equivalent to one third of a tennis court. It is at least twice as efficient as conventional coconut-derived carbon at absorbing toxic volatile compounds from cigarette smoke in laboratory tests[3].

The new carbon is derived from a polymer that is carbonized by heating. The resulting carbon has a pore structure that is not accessible for typical adsorptives. However, further heating produces a microstructure with pore sizes from 0.7–3 nm, and activation with carbon dioxide leads to the formation of slightly larger pores from 3–80 nm.

The larger pores act as 'transporters' that guide the toxicants towards the smaller pores where they become trapped.

The smoke travels through the filter at speed. Without the larger transport pores to guide the toxicant molecules into the smaller pores, getting them in there would be a bit like trying to park a car at right angles while driving at speed.

Tests using 60 mg (equivalent to 1/70th a teaspoon of sugar) of carbon in the cigarette filter can reduce the levels of certain smoke toxicants by as much as 60%, compared with existing coconut-shell derived carbon.

The second filter technology is called CR20 — a macroporous polystyrene-based ion-exchange resin. It is produced by the Mitsubishi Chemical Company and is a modified version an ion-exchange resin commonly used for waste-water treatment because of its affinity for transition-metal ions. The material usually comes in the form of beads in an aqueous environment.

To make it suitable in a cigarette filter application, the water is removed and the material dried to around 15% or less moisture.

The resin is weakly basic and is cross-linked with divinyl benzene. It offers the potential for the nucleophillic capture of aldehydes from mainstream smoke by forming enamines (1).

1. $RCHO + R'NHR'' \rightarrow RC=NR'R'' + H_2O$

Due to its weakly basic nature, it can also be used for the removal of hydrogen cyanide (HCN) from the cigarette smoke (2 and 3).

2. $R\text{-}NH_2 + HCN \rightarrow R\text{-}NH_3 + CN^-$

3. $R_2NH + HCN \rightarrow R_2NH_2 + CN^-$

Using 60 mg of resin beads in a cigarette cavity filter, we investigated its ability to react with aldehydes and HCN in the cigarette smoke. Smoking experiments confirmed that CR20 is selective and highly efficient for the filtration of certain aldehydes (in particular formaldehyde) and HCN in cigarette smoke[4].

The results showed substantial reductions in smoke formaldehyde of greater than 50% (estimated to be up to 80% of the formaldehyde present in the smoke vapour phase) independent of smoke flow rate. Substantial reductions of HCN (>80%) and acetaldehyde (>60%) were also observed. The reductions in these compounds were greater than those achieved using a microporous active carbon based on a physiosorption mechanism.

These results are very promising, but tests with smoking machines have their limitations. The results can give an indication as to whether a technology can reduce toxicants in smoke, but they cannot tell us whether this will have an effect on smokers' exposure to these toxicants and whether reducing that exposure has any biological relevance. For that we need to do biological testing in the lab using cells and monitor smokers smoking these prototypes in the clinic (see pages 10-12).

1. Liu, C *et al.*, The use of a novel tobacco treatment process to reduce toxicant yields in cigarette smoke, *Food and Chemical Toxicology,* 2011; **49**, 1904–1917.
2. McAdam, K. *et al.*, The use of a novel tobacco-substitute sheet and smoke dilution to reduce toxicant yields in cigarette smoke, *Food and Chemical Toxicology,* 2011; **49,** 1684–1696.
3. Branton, P. *et al.*, and Bradley, R., Effects of active carbon pore size distribution on adsorption of toxic organic compounds. *Adsorption* 2011, **17,** 293–301.
4. Branton, P. *et al.*, Reduction of aldehydes and hydrogen cyanide yields in mainstream smoke using an amine functionalised ion exchange resin, *Chemistry Central Journal* 2011, **5,** 15.

Choosing a candidate technology

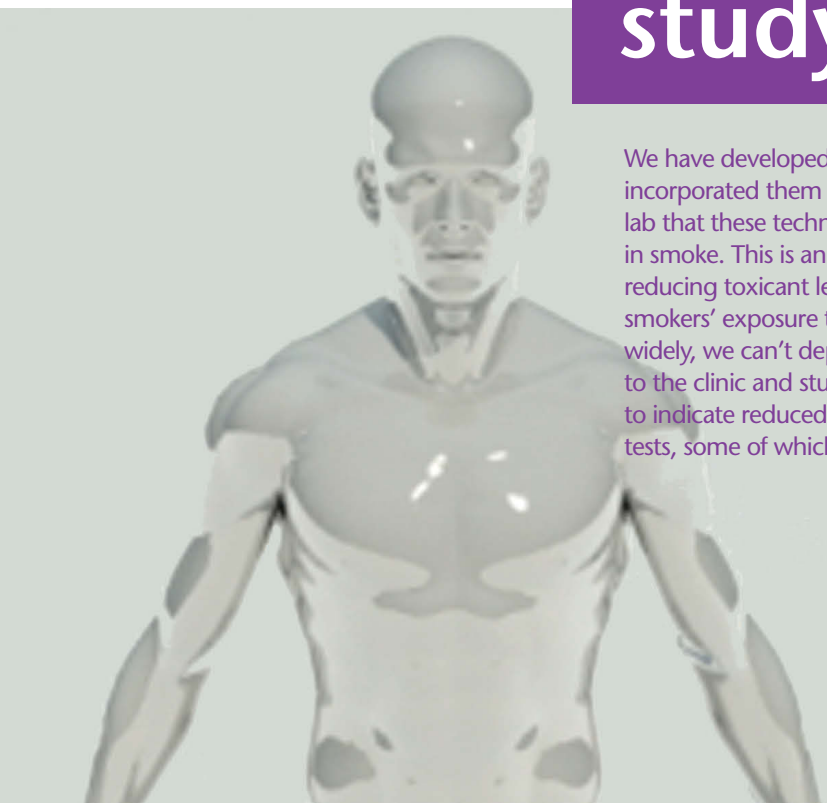
There are a number of issues that commonly arise when new candidate materials are being considered. Some materials are simply rendered ineffective by the smoke; and because the materials are burned in the cigarette there is also the potential for the generation of new compounds which could be toxic.

In terms of filtration, two issues commonly arise when we investigate new filter additives or materials. The first is that as cigarette smoke is highly complex (containing thousands of known compounds), the additives / materials are often poisoned or coated by tar in the first puff rendering them ineffective. The second is that the residence time in filter is typically very short (<0.01 secs) and as such, the cigarette smoke has passed through the filter before it has an opportunity to impact the smoke.

Another area of attrition in the development of technologies is the ability to incorporate it into a cigarette and deliver a sensorially acceptable smoke. In some cases it is possible to design the blend and cigarette to offset a small reduction in sensory performance, but in others it is not without reducing the efficacy of the technology.

The scale-up stage brings its own challenges and may be the longest stage in the development of a new technology (in some instances up to twenty years) to meet high speed/volume production needs.

Our first clinical study results



We have developed some promising toxicant reducing technologies and incorporated them into prototype cigarettes. And we demonstrated in the lab that these technologies do successfully reduce the levels of toxicants in smoke. This is an important step, but we need to know whether reducing toxicant levels in cigarette smoke has the effect of reducing smokers' exposure to these toxicants. Because smoking behaviour varies widely, we can't depend on laboratory data to tell us this, we have to go to the clinic and study groups of smokers. But even this is not enough to indicate reduced risk. For that, a wide range of scientific studies and tests, some of which are still being developed, will be needed.

Dr Christopher Proctor
Chief Scientific Officer

Research over many years has shown that the way people smoke, called smoking behaviour, varies widely from person to person, and that the interaction between a smoker and their cigarette can affect exposure to toxicants. Moreover, changing the cigarette, either in the way it functions or in the sensory properties of the resulting smoke, can impact the way someone smokes a cigarette and ultimately their exposure to toxicants in the smoke.

So while laboratory tests on the chemistry of the smoke from modified cigarettes can indicate the potential of a new product, studies using biomarkers of exposure are the only way to determine whether and to what extent there is toxicant exposure reduction in a group of smokers. Biomarkers, which we have measured in blood, urine and saliva, are the toxicants themselves or their metabolites. Generally the higher the levels found in urine or saliva, the greater the smokers' exposure to the toxicant, depending on individual metabolic differences.

The half-lives of most of the key toxicants in tobacco smoke are relatively short – typically a matter of hours or days – and so clinical studies aimed at determining toxicant reductions can be relatively short-term studies. However, because of the potential changes in smoking behaviour as a smoker gets accustomed to a different style of cigarette, we consider studies that last around a month after switching to be appropriate.

In vitro assessment of modified cigarette smoke

Laboratory based tests using human cell systems are used to determine whether the modifications we have made to the cigarettes are biologically relevant. In general, we investigate the changes in disease-related mediators at the protein and gene level using an array of commercially available and in-house developed technologies, including multiplex ELISA (Meso Scale Discovery® platform), SuperArray and Affymetrix® Gene Chip technologies. Our aim is to develop a series of high-throughput tests that more accuately reflect the complex processes involved in the development of tobacco-related diseases, such as chronic obstructive pulmonary disease, cardiovascular disease and lung cancer, as well as oxidative stress and inflammatory processes.

One of the challenges is to present the biological system with smoke in a form relevant to what smokers are exposed to. There are three main sampling approaches that sample different forms of cigarette smoke:

Particulate matter: The particulate phase of cigarette smoke is trapped using Cambridge filters pads and extracted using a solvent. This type of cigarette smoke has been traditionally used for routine *in vitro* toxicological testing.

Aqueous extracts: Cigarette smoke is bubbled through biological buffers or cell culture media, capturing the water-soluble components of the particulate and gas phase.

Whole smoke: All phases of the cigarette smoke can be assessed using an in-house developed *in vitro* exposure device. We are working closely with other industries to further demonstrate and assess applicability of the technology for other individual and complex aerosols.

Toxicant exposure will be affected by the smoking behaviour of an individual smoker. The most important determinant of toxicant exposure is likely to be the number of cigarettes smoked each day. In addition, though, a smoker that takes more puffs from each cigarette or takes large puffs is likely to increase toxicant exposure. Then, of course, there will be the effect of the smoke chemistry of the product itself. To understand the potential for reduced toxicant prototype cigarettes it is necessary to study both the performance of the product and its interaction with a group of smokers.

We recently conducted our first clinical study of modified prototype cigarettes and we have shown that it is possible to reduce smokers' exposure to certain smoke toxicants[1, 2].

In the Clinic

Our study was a single-blinded randomised controlled study conducted over a 6-week period, in Hamburg, Germany[2]. But before we went to the clinic, we conducted *in vitro* tests to determine whether the reductions in smoke toxicant levels we achieved with the prototypes in the lab are biologically relevant (see box).

In the clinical study, three prototypes were studied, see figure 1. Each prototype included several of the four toxicant reducing technologies described on pages 7-9. The other products in the study were conventional cigarettes based on products popular in Germany.

The majority of the subjects were regular adult smokers, judged to be healthy in a screening visit to the clinic. A group of 50 non-smokers were also studied in order to measure the background level of the biomarkers of exposure not associated with smoking but originating from another source of exposure either in the diet or from the environment.

There were around 250 smokers, 100 of which were regular medium ISO tar smokers. These subjects were randomly assigned to one of two groups; one group smoked a conventional cigarette throughout the study while the other group switched to a reduced toxicant prototype after two weeks. The other 150 subjects in the study were regular very low ISO tar yielding cigarette smokers and these were randomised into three groups of 50, one group smoking the conventional cigarette throughout and the other two groups being switched to one of two reduced toxicant prototypes. The study was single blinded, meaning that the researchers knew which subject had the test and which had the control products but the subjects themselves did not know.

All of the smokers smoked conventional cigarettes for the first two weeks of the study. At that time they were asked to come to the clinic and stay for two days, including overnight. During this time they collected all of the urine that they passed. The urine was later analysed for levels of the biomarkers of exposure. Twenty-four hour collections of urine are thought to be better than single spot samples, as exposure and excretion varies at different parts of the day. While in the clinic, the smoking behaviour of the subjects was observed for their smoking behaviour patterns. The cigarette filters were collected to see how intensely they were smoking, and they were asked to complete a questionnaire on sensory aspects of the cigarettes. At this point in the study, some of the smokers were switched to the reduced toxicant prototype.

Subjects visited the clinic on two more occasions, first after four weeks (i.e. those who had been switched had been smoking the prototypes for two weeks) and again at the end of the study at 6 weeks.

The urine was sent to biochemical laboratories where it was analysed for levels of a wide range of biomarkers of exposure. In some cases, such as with nicotine and one of the tobacco-specific nitrosamines, the biomarker was a metabolite or a series of metabolites of the original compound, whilst in other cases it was levels of the compound itself. A wide range of toxicants were covered in the study, and included vapour phase irritants such as acrolein and particulate phase carcinogens such as tobacco specific nitrosamines and 4-aminobiphenyl.

The results showed that certain toxicant exposures were reduced, as assessed by biomarker of exposure levels, in the groups switched to the reduced prototype cigarette compared to the group that continued smoking the conventional cigarette, see figure 2. For vapour phase toxicants such as acrolein and 1,3-butadiene reductions of ≥70% were typically observed both in smoke chemistry and biomarkers of exposure. Reductions in particulate phase toxicants such as tobacco-specific nitrosamines, aromatic amines and polynuclear aromatic hydrocarbons depended upon the technologies used, but were in some cases ≥80%.

The reductions in levels of toxicant exposure were in the main seen two weeks

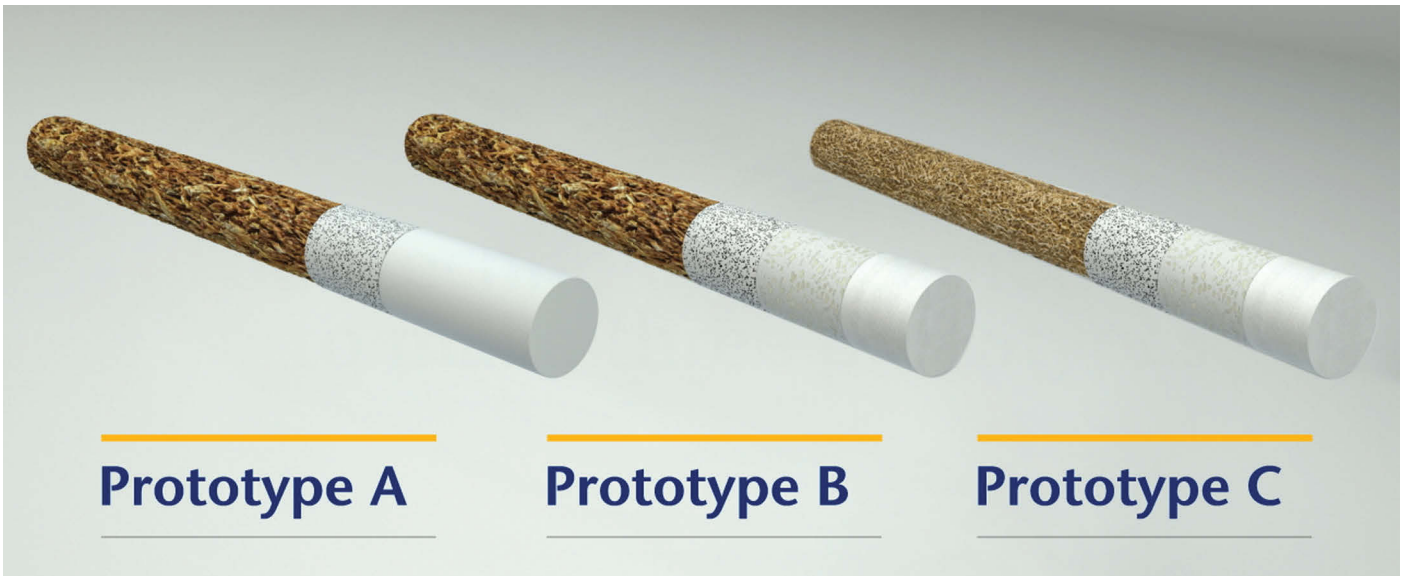


Figure 1. Three prototypes were created using different combinations of four technologies: an enzyme-treated tobacco, a glycerol-based tobacco diluent, a nanocarbon filter and an ion-exchange resin.

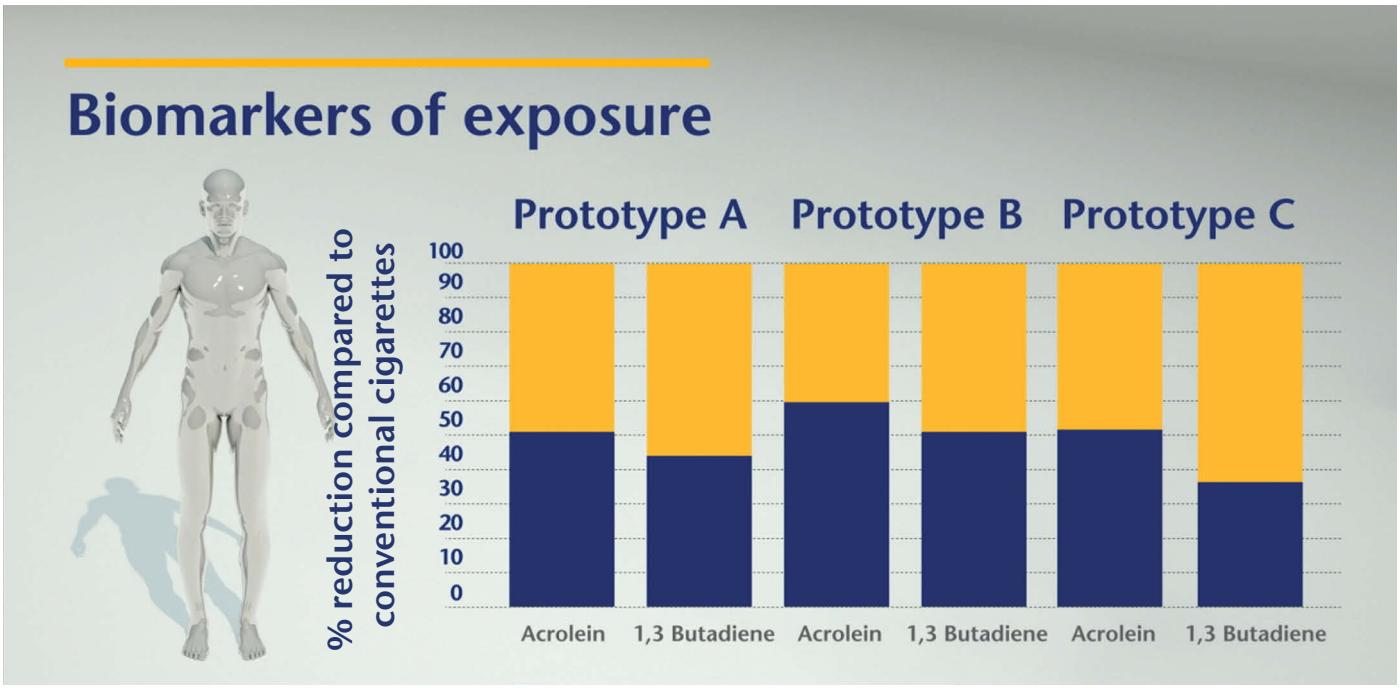


Figure 2. Smokers of prototypes had statistically significant reductions in exposure to acrolein and 1, 3 butadiene - reductions were greater and sometimes much greater than 50 per cent.

after switching and were maintained through to the end of the study. The reductions corresponded to the technologies used in the prototypes – there was always a reduction in vapour phase toxicants but in some cases no reduction, or increases, in some of the particulate phase toxicants not addressed by the technologies. The levels of the biomarkers of exposure were always lower, and sometime much lower, in the non-smoker group, illustrating that even when toxicant exposure was reduced it was not eliminated, by switching to the reduced toxicant prototypes.

The study illustrated the methodological difficulty in assessing such products. In some cases, cigarette consumption increased, which could be a consequence of the subject being in a clinic being given cigarettes on request.

When analysing the data individual by individual, it is clear to see how challenging it would be to be able to claim that a reduced toxicant prototype reduces toxicant exposure, not just on average for a group, but for every individual in the group.

It is also possible to use the clinical data to see which smoking regime method in the laboratory best mimics on average the amount of toxicant exposure likely in groups of smokers. This is an issue being considered by regulators, and any regulations seeking to mandate toxicant reductions will have to determine an appropriate smoking machine regime.

The study was too short to consider whether the reductions in toxicant exposure might have an impact on health risks. That would require a longer study of at least six months and a wide range of biological tests, including studies of validated biomarkers of biological effect. As a next step, a longer-term clinical study is currently underway[2].

1. Clinical Study Paper (tbc)
2. http://www.controlled-trials.com/ISRCTN72157335
3. http://www.controlled-trials.com/ISRCTN81286286/British+american

REGULATION

A regulatory approach to reduced toxicant products

By Dr Christopher Proctor

Regulatory oversight of tobacco harm reduction is particularly important for a number of reasons. Several uncertainties remain in evaluating the risk profiles of tobacco products designed to reduce risk, and there are also uncertainties about future market dynamics; for example, would reduced-risk tobacco products be especially attractive to non-tobacco users or would their availability reduce the likelihood of quitting in existing tobacco users? The challenge for regulators is to build sufficient scientific expertise and surveillance capability to successfully evaluate the potential impact of new products on both individual and population risks.

To date, the only regulator to rise to the challenge is the US Food and Drug Administration (FDA). In 2009, it was given authority under new legislation to regulate tobacco products in the USA, including the evaluation of submissions on candidate 'modified-risk tobacco products'. In developing a regulatory framework, the FDA worked with other departments to form an Office of Science with a multidisciplinary team lead by an experienced and well-published tobacco researcher. The Office set out guiding principles, including a reference to the importance of science in informing regulatory decisions. It has sought to bring a broad church of scientific advice to promote policy development and employed several different approaches.

In 2011, the FDA asked the US Institute of Medicine (IOM) to form a panel of experts to produce a report on scientific standards for studies of modified-risk tobacco products. In 2001, a previous IOM panel produced the seminal report on tobacco harm reduction (*Clearing the Smoke, Assessing the Scientific Base for Tobacco Harm Reduction*, 2001, National Academy Press, Washington DC, US). The FDA also engaged a multidisciplinary Tobacco Products Scientific Advisory Committee to look at and report on scientific issues related to a series of subjects, including menthol as an ingredient to cigarettes, constituents of tobacco and tobacco smoke that are harmful or potentially harmful, as well as dissolvable tobacco products. The committee operates transparently with public meetings, webcasts and opportunities for public comment. It also includes nonvoting representatives from various parts of the regulated industry. In addition, the FDA held a two-day public workshop with a range of experts discussing the science underpinning modified-risk tobacco products, and encouraged a wide spectrum of interested parties.

In 2012, the FDA issued guidance on the reporting of 93 harmful and potentially harmful tobacco and smoke constituents, of which an abbreviated list of 20 constituents are to be reported initially. In addition, the FDA has the authority to adopt product standards that may require the reduction or elimination of certain constituents, i.e. to introduce limits.

Outside of the US, tobacco regulation globally is steered by the World Health Organization (WHO), which guides tobacco control by national governments under the Framework Convention on Tobacco Control. The framework convention does not mention reduced risk tobacco products, let alone set out how to regulate them. The WHO does have an advisory scientific committee — the Study Group on Tobacco Product Regulation (TobReg) — and this expert group has produced a series of reports, including one which concluded that smokeless tobacco products present lower risks than cigarettes. It has also suggested regulatory controls over toxicants in cigarette smoke. To date, however, the WHO has yet to take TobReg's scientific advice or apply it to create practical guidelines for national regulators on this issue.

There is great potential for public health gains if a regulatory framework could, in addition to supporting the broader public health goal of prevention and cessation of tobacco use, encourage adults refusing to quit to transition to substantially less risky products. With Swedish style snus, for example, there is epidemiology to determine their risk profile, allowing regulators to act. Furthermore, particular smokeless products are known to present higher risks than others on the market. Therefore, setting product standards based on the epidemiology of different products and specific toxicant levels might make sense.

For cigarettes, however, the scientific framework to determine whether lower levels of smoke toxicants will result in a lower risk profile is incomplete. As such, it is not possible to conclude that mandated toxicant reduction results in less risky products. Nonetheless, this may still be a viable regulatory approach as long as the scientific basis is agreed. This would involve determining which toxicants or groups of toxicants are the most important, what the dose response curves might look like for these toxicants and various diseases, and whether reducing a set of toxicants might produce some unexpected health risks. Such a research agenda, we believe, should be encouraged by regulators and undertaken by a consortium of scientific interests.

COLLABORATIONS

We have a number of research collaborations with academic and industrial partners, as well as with specific trade organisations, regulators and, in some areas, local governments. Most of our collaborations focus on our research efforts in support of tobacco harm reduction.

PLANT BIOTECHNOLOGY

In the area of plant biotechnology we have agreements with several internationally recognised groups. We hope that these collaborative projects will lead to advances in our understanding of the growing tobacco plant, in order to determine whether it is possible to reduce toxicants in the leaf or reduce the levels of toxicant precursors in the leaf. We have agreements with groups such as Keygene and the Max Planck Institute.

The collaboration with KeyGene, the Netherlands, is focused on major harm reduction targets and crop sustainability. This utilises KeyGene's technologies in crop improvement, which have found application in one third of the entire vegetable seeds produced world wide. KeyGene apply bioinformatics and systems biology analysis to explore natural genetic diversity within a crop to identify specific genes that could impact desirable characteristics and accelerate breeding.

Another major collaboration is with the Max Planck Institute for Molecular Plant Physiology, Germany. This internationally recognised institute in plant science is using its expertise in plant metabolomics to study chemical profiles in tobacco leaf. The study will identify molecules in tobacco leaves that are precursors for toxicants in cigarette smoke. These precursors will then be used as targets in research programs to breed new varieties of tobacco with reduced levels of toxicants.

TECHNOLOGY AND ANALYTICAL SCIENCE

We have a number of collaborations to support the development and assessment of technologies to remove toxicants from tobacco smoke and analytical methods to measure toxicants in tobacco smoke.

For example, activated carbon is an excellent adsorbent material that can be used to reduce the yields of many vapour phase toxicants in cigarette smoke. Together with Blucher GmbH, we have synthesized a novel activated carbon with a porosity tailored to maximise the reductions in many of these toxicants (see page 8). Work has continued with other academic and industrial partners to use this unique porosity in activated carbon derived from different more sustainable carbon sources.

Our collaborations in the field of analytical science include a joint research project with the Centre for Analytical Research and Technology at the University of Liège, Belgium to apply two-dimensional gas chromatography and Time-of-Flight mass spectrometry to identify and measure large numbers of substances in tobacco smoke. We are also collaborating with the Food and Environment Research Agency (part of DEFRA, UK) to evaluate the application of non-targeted Nuclear Magnetic Resonance spectroscopy to the measurement of tobacco smoke toxicants and to develop advanced methods for the characterisation of processed tobacco.

Dr Marianna Gaca
Engagement and
Collaboration Manager

BIOLOGY

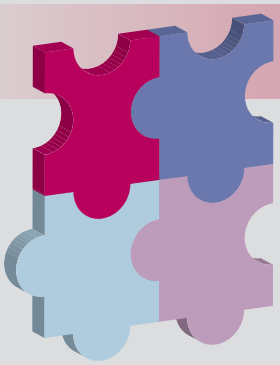
We also have a number of successful collaborations on characterising the biological effects of cigarette smoke. We have for example worked on collaborative projects with the University of North Carolina, USA; the University of Milan, Italy; the Russian Academy of Science; BioMed zet Life Science, Austria; and Epithelix Sárl, Switzerland.

In collaboration with the University of North Carolina, we have developed a biological tool to study the early pathways of cigarette-smoke lung disease mechanisms. A protein, called NFkB, is known to be a first responder when cells are exposed to harmful external stimuli such as environmental chemicals. This acts to switch on the genetic material required to initiate an immune response. We have modified this protein so that it is labelled with a fluorescent dye, and can be incorporated into cell cultures. We are able to track the activity of this protein in real-time using rapid and automated laboratory detection systems. This should allow us to understand its contribution to inflammation following exposure of cells to tobacco smoke and to gain a better understanding of the mechanisms involved in disease progression.

In addition and in collaboration with Caprion, a contract research organisation in Canada, we have been able to identify and quantitate up to 100 proteins, at the same time, in the biological liquid that covers the surface of cells found in the lung. It is hoped that this will allow us to monitor disease progression and smoke-induced injury much quicker using both sputum samples from patients and *in vitro* models being developed in Southampton.

At the University of Milan we are investigating the effects of cigarette smoke on the development of atherosclerosis, and are focussing on human white blood cells called monocytes and macrophages. From these studies we hope to be able to develop an *in vitro* test that will allow us to assess the potential of our products to contribute to cardiovascular disease.

A number of studies have also been undertaken with the Russian Academy of Sciences and includes the successful development of sensitive detection tools and methods to analyse free radicals in tobacco smoke and cellular *in vitro* systems. These free radicals are responsible for causing damage to DNA, proteins and fats inside cells, causing cells to become dysfunctional which leads to disease. These tools will enable us to understand the formation of free radicals in tobacco smoke and during disease processes.



CASE STUDY

We have been involved in a collaboration on *in vitro* cancer research since 2010, involving BioMed zet Life Science, a company specialising in the development of *in vitro* assays, based in Linz Austria and Epithelix Sarl, a biotech startup based in Geneva, Switzerland.

The aim of this collaboration is the development of an *in vitro* test for cancer using a unique 3-D 'lung in a lab' model designed to assess the effect of tobacco smoke and other environmental chemicals on the human airway.

The model under development is based on a reconstituted human lung tissue technology called MucilAir™, developed and provided by Epithelix.

MucilAir™ is unique as it has a shelf life of over a year, allowing long-term and repeated testing – most commonly used tests have a shelf life of just a few weeks.

The human airway is lined with an epithelium which has a complex 3-D structure of many diverse cell types which is physiologically mimicked with MucilAir™ cultures. The model can be used to evaluate the effects of smoke on healthy cells, those of ex-smokers and those of smokers. In the future, the same model could be used to test prototype tobacco products designed to reduce risk. In addition, the model could be used for the testing of environmental chemical or pharmaceutical therapies at different stages of lung disease without animal experimentation.

Dr. Klaus R. Schröder CEO BioMed-zet Life Science GmbH and Chairman of zet Centre for Alternative and Complementary Methods to Animal Testing

The science project we have started is a unique international collaboration between Great Britain, Switzerland and Austria.
Cell culture techniques are widely used to study health effects. Until today they have been restricted to the short life time of the systems. They only allows for short term studies of acute health effects. In our project we analyse a long-living lung tissue system, called MucilAir™, from Epithelix Sárl. It remains viable for more than one year. This allows the analysis of long-term chronic effects with compound concentrations that are generally regarded as sub-toxic. In this respect, we are spearheading *in vitro* research in this area.

Although the project involves a certain amount of fundamental research in analyzing the inherent capacities of the lung tissue systems, we can already forsee several important potential applications. For example, a new long-lived *in vitro* model for the lung should have a major impact on the study of those diseases that manifest only after long exposure to low substance concentrations, i.e. cancer.

To date, animal models are most commonly used in the study of these diseases. We hope that, in the future, the models we develop will provide an alternative to existing animal models, which are extremely time consuming and expensive. Our system will shorten the time taken to obtain results and cut costs substantially. Once the system is established, it could also find application in the testing of pharmaceutical anti-cancer drugs and environmental chemicals *in vitro*. It offers the opportunity to study molecular cancer mechanisms without animal experiments.

In our view, British American Tobacco (BAT) puts strong emphasis on the science of tobacco health reduction. Over many years, BAT scientists have acquired the expertise to perform cell culture research that is exemplary for the study of lung toxicity effects.

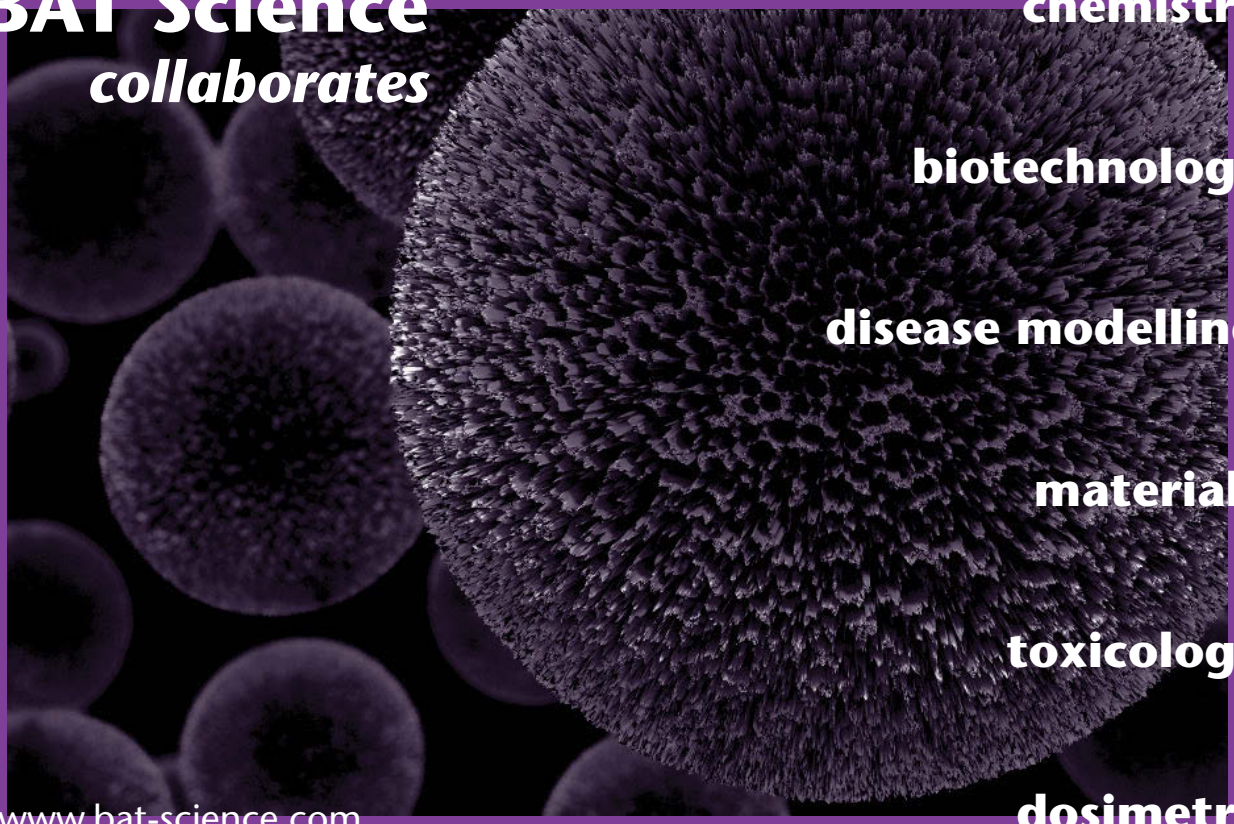
We are a non-profit research organization that seeks to establish and characterize cell and tissue culture systems for industrial research and development applications.

We had decided to characterize the lung tissue system MucilAir™ for its capabilities in the field of cancer research prior to our collaborating with BAT. However, when we became familiar with BAT's research activities, we identified not only a common interest in fostering *in vitro* technologies in applied research, but also a perfect opportunity to contribute to BAT's research towards tobacco harm reduction. BAT's long experience in cell culture, their knowledge in physiological mechanisms in lung toxicity combines nicely with our research concept, and has lead to a fruitful collaboration.

Artist's impression of human airway epithelia, MucilAir™.

BAT Science

collaborates



chemistry

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toxicology

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