



Photo: BAT

## Reference products used in tobacco and smoke analyses

Reference and monitor products have been an important foundation of tobacco product scientific research for many years. Reference cigarettes are used in many ways, for example, in the development and validation of analytical chemical and toxicological testing methods, as internal laboratory controls in on-going analytical work and as a common factor during the comparison and interpretation of results from different laboratories.

Users include tobacco product manufacturers and tobacco product testing laboratories, producers of packaging and analytical testing machines, laboratories, hospitals, medical centers, research institutes, colleges and universities. Many scientific papers, too numerous to discuss individually, publish constituent levels, in-

clude smoke yield and toxicity data obtained during novel method development or studies on a range of products that regularly include these reference materials.

As the main suppliers, both the University of Kentucky and CORESTA have played key roles in supporting the tobacco research community by provid-

ing reference cigarettes and monitor test pieces respectively. In addition, CORESTA have also recently organised the production of four new reference smokeless tobacco samples.

Such products are made to fulfill requirements for reference materials, such as those given in ISO Guide 30, which states that they should be suffi-

ciently homogenous and have measurement values that are sufficiently well established so that they can be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

### Kentucky Reference Cigarettes

The University of Kentucky has provided reference cigarettes for 45 years as a service for tobacco research both within the United States and globally. During the late 1960's when increased emphasis was being placed on smoking and health research, it was determined that a cigarette that could be used as a research standard should be developed. The first reference cigarette, the 1R1, was produced in 1968, and since then various reference cigarettes have been produced and distributed. Specific blends were developed in conjunction with the United States Department of Agriculture, the National Cancer Institute, tobacco industry representatives and the University of Kentucky. Kentucky reference tobacco products were designed to represent the tobacco composition of US consumer tobacco products but do not include any additional flavorings.

The reference cigarettes are made under contract by manufacturers specifically to be held by the University of Kentucky prior to sale to tobacco researchers. They are produced under normal production tolerances so that their variability is similar to commercial products and are made in quantities large enough for the same reference cigarette to be used over a time period of several years. The packaging states that the contents are research cigarettes to be used only for tobacco-health research and experimental purposes, and shall not be offered for sale, sold, or distributed for commercial or consumer use.

Since 1968, there have been 17 reference tobacco products produced and distributed through the University of Kentucky including mostly reference cigarettes (14) but also three smokeless tobacco reference products. Currently

there are commercial level stocks of two reference cigarettes, the 1R5F and the 3R4F with approximately 400,000 1R5F and 28 million of the 3R4F remaining. The 1R5F is a low yield cigarette (approximately 1.7 mg/cigarette nicotine-free dry particulate matter (NFDPM) and 0.2 mg/cigarette nicotine as determined under the ISO smoking regime conditions) while the 3R4F was designed to be more representative of the most popular products on sale in the US cigarette market (approximately 9.2 mg/cigarette NFDPM and 0.8 mg/cigarette nicotine). In addition, there are small numbers of 11 of the older types which may be obtained for specific research projects depending on availability and need. Further types to reflect the range of commercial products will become available in the future.

For information, the cigarette acronym (e.g. 1R5F) is derived as follows: 1 = First production of a particular cigarette version; R = Reference Cigarette; 5 = 5th in a series of blend/design versions; F = Filtered.

During production, the goal is to produce enough of a particular version to last 10 years. The production amount will vary depending on the use and need. When there is another run of a particular version every effort is made to keep the blends and production para-

eters consistent with the previous version. However, tobacco is an agricultural crop so there is no way to exactly match the two runs, but they are blended and designed to be as close as possible. The goal is to use the same types of tobaccos in the blend but they are from different crop years and so some compositional differences will occur. In addition, some tobaccos may no longer be available. With each batch of reference cigarettes, a preliminary assessment is made to ensure the contract parameters have been met such as physical testing specifications throughout production and analytical yield targets. There is a need to perform well designed analytical studies to characterise the reference cigarettes for both levels of blend and smoke constituents and to derive the measurement precision within and between laboratories. This is done through collaborative studies, most often through CORESTA, using harmonised, standardised methods. However, some scientific studies within individual laboratories providing constituent and yield data are provided on the reference cigarette website. The University of Kentucky is investing in additional analytical capability for in-house reference cigarette analysis and quality control to provide additional information.

Reference cigarettes are stored at -260C in a bonded warehouse until being shipped to fulfil orders. Over the past 5 years, Kentucky reference cigarettes have been shipped to 40 countries. The highest number of orders come from universities and research organisations although these are generally for very small numbers of less than 5000 cigarettes.

### CORESTA Monitor test pieces

In contrast to Kentucky reference cigarettes, CORESTA manufactures monitor test pieces made from flue-cured lamina tobacco only, containing no tobacco stems, reconstituted tobacco sheet or expanded tobacco and no non-tobacco ingredients. A relatively small batch (5 million) of the most recent test piece



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Photo: SWM

(CORESTA Monitor 7, CM7) was manufactured within a 2 day production period at a relatively low production speed with a tighter weight tolerance than the Kentucky reference cigarettes according to the provisions given in ISO 16055. Thus any measurement differences are less likely to be due to product variability and can be used to monitor instrument performance over time and provide critical performance data for analytical method development. The greater uniformity of these products means that they are particularly useful in quality control to monitor the stability of the analytical processes involved when using a cigarette smoking machine for routine analysis. In particular, they can be used to assess whether the analytical process related to the machine smoking of cigarettes is in statistical control. In this way the CORESTA monitors both add additional value and complement Kentucky reference cigarettes.

Six test pieces (CM2 to CM7) have been produced since 1995 by manufacturers on behalf of CORESTA according to the provisions given in ISO 16055. Guidelines are given on the CORESTA website under "Technical Documents" providing details on specification, production, packaging and evaluation. Further de-

tails on the latest monitor CM7 are also given on the same website in the document entitled "CORESTA Approved Monitor No. 7 (CM7) Use and Conditions". Samples can be ordered from Borgwaldt or Cerulean.

In 2010, ISO 12863 was published which described a laboratory test to measure the percentage of cigarettes that self extinguish under standardised conditions and which according to some regulatory authorities gives a measure of the ignition potential of cigarettes. The requirement to pass the test is that no more than 25 per cent of a batch of cigarettes should burn through their whole length. The US National Institute of Standards and Technology (NIST) has produced a Standard Reference Material (SRM 1082) for this test which is very different from commercial products, being made from 100 per cent expanded tobacco and non-banded cigarette paper. It was tested prior to release by three laboratories and a certified value for its performance in the test was determined. Looking to the future, CORESTA is currently working on the production of Low Ignition Propensity reference cigarettes which will use banded paper typical of commercial products and which will provide different performance levels under this ISO standard.

### CORESTA smokeless tobacco reference products

Since there is a large range of smokeless tobacco products on sale globally there is a need for reference materials to enable comparisons between different sets of analytical data. The North Carolina State University Tobacco Analytical Services Laboratory (TASL) serves as the central repository for Smokeless Tobacco Reference Products. Early reference products produced in this programme included the 1S1 and 2S1 (loose leaf chewing tobacco), 1S2 (dry snuff), 1S3 and 2S3 (moist snuff) products and some product characterisation data is provided on their website. More recently, the TASL has been working with members of the CORESTA Smokeless Tobacco Sub-group to provide a new series of standardised smokeless tobacco reference materials. They were intended as fresh replacements for 2S3, 1S2 and 2S1 which were greater than ten years old. These four products are called CORESTA Reference Products (CRPs) relating to different styles, that is, CRP1 (Snus), CRP2 (moist snuff), CRP3 (dry snuff), and CRP4 (loose leaf chewing tobacco). These materials are stored at -18 °C and distributed by the North Carolina State University To- ▶

bacco Analytical Services Laboratory. In order to characterise these CRPs the CORESTA Smokeless Tobacco Sub-group organised analyses of some of the major components and some of the constituents of current regulatory interest. Since few standardised methods are available currently, individual laboratory methods were used and the data demonstrated a wide range of values. A report on the product characterisation is expected to be made available on the CORESTA website in due course. Future studies of this nature will incorporate specified methods for each analyte since CORESTA is developing many recommended methods (CRMs) through this Sub-group. CRMs have already been published for sampling, 'pH' and tobacco specific nitrosamines (TSNAs). The stability of these reference products will be monitored annually for selected analytes ('pH', oven volatiles and TSNAs). A CRM for the manufacture of these reference products will also be produced.

### Some collaborative studies in which reference and monitor cigarettes have been used

Collaborative studies are useful to laboratories in order to maintain their accreditation status for the methodologies and demonstrate data validation through ISO 17025. Commercial samples are chosen within particular studies but the inclusion of reference cigarettes allows not only comparisons between laboratories within a study but also comparisons between data generated in different studies.

The CORESTA Routine Analytical Chemistry Sub-group (RAC) runs annual smoke testing collaborative studies to obtain mean yields of NFDPM, nicotine and carbon monoxide (CO) from the CORESTA Monitors and to derive within-laboratory variability (repeatability "r") and between-laboratory variability (reproducibility "R"). The most recent studies now include the collection of data under both the ISO 3308 and the Health Canada Intense (HCI, Health Canada Regulations, 1999) smoking re-

gimes. Variability data from both reference and commercial cigarettes was used to set the measurement tolerances given in ISO 8243 for tar, nicotine and CO yields.

The Asian Collaborative Study (ACS), organised by the Tobacco Institute of Japan, has been running for 20 years and currently involves 57 laboratories from 26 countries. As well as the inclusion of several commercial cigarettes, the CORESTA monitors have been included, starting with CM2 in 1995. The ACS reports NFDPM, nicotine and CO smoke yields and variability under the ISO 3308 smoking regime compared with data from the CORESTA RAC studies.

There are also annual studies run in Europe organised by the Deutscher Zigarettenverband (DZV, the German cigarette manufacturers association). For example, the 2010 study involved 46 laboratories smoking 5 products, including the CM6 test piece, under the ISO 3308 smoking regime.

Additional collaborative studies have also taken place such as the 2010 study organised by ISO/TC126 Working Group 10, involving 35 laboratories that produced NFDPM, nicotine and CO smoke yield data on 10 products, including the CM6 and 1R5F products, and also a 2008 study organised by the Tobacco Laboratory Network (TobLabNet) of the World Health Organisation, involving 14 laboratories who similarly analysed five cigarette types including the CM6, 1R5F and 3R4F products.

Both the CORESTA monitor test pieces and the Kentucky reference cigarettes (3R4F and 1R5F), alongside a range of commercially available cigarettes, have been used by the CORESTA Special Analytes Sub-group in the development of CRMs for the measurement of yields of benzo[a]pyrene, tobacco-specific nitrosamines, selected volatiles and selected carbonyls in mainstream smoke (CRMs 58, 63, 70, 74 and 75 respectively) which are available on the CORESTA website. These CRMs include mean yields, repeatability and reproducibility data for these components, obtained under both ISO and the HCI smoking regimes. Currently work has moved on to

developing methods for selected phenolic compounds and ammonia in cigarette mainstream smoke.

The CORESTA In Vitro Toxicity Testing of Tobacco Smoke Task Force have also used the 2R4F and 3R4F reference cigarettes in a 2003 inter-laboratory study of cigarette smoke condensate from four cigarettes to compare data for the Ames, Neutral Red Uptake and Micronucleus assays. Later collaborative studies in 2008 on the Ames assay; 2009 on Neutral Red Uptake and 2010 Micronucleus test also used the 3R4F cigarettes.

### Regulatory issues

Currently, there are no regulations that require the inclusion of reference products in any testing of tobacco or cigarettes. The importance of reference products in the regulatory environment may increase in the future. In 2012 the US Food and Drug Administration (FDA) requested the reporting of yields of 20 smoke components for all products on sale in the USA. However, since the FDA did not specify that standardised methodology should be used to obtain the data and did not request the inclusion of any reference cigarettes it is difficult to see how the data from different manufacturers might be compared. Reference cigarette data and standardised methodology should help to prevent the unintended consequences of data misinterpretation.

On 11-12th April 2012, the FDA Centre for Tobacco Products organised a Scientific Public Workshop on Tobacco Product Analysis during which all of these issues were discussed. There were presentations on the availability, design, manufacture, storage, stability, characterisation and uses of reference products and open discussions on current and future needs. It is encouraging to find that the FDA recognises the potential worth of data on reference products and the need for continuing dialog between various stakeholders since the intention should be that future regulatory decisions by FDA or in other global regions should be based on sound science.

**TJI Report**