Complementary information to the CORESTA presentation in Washington DC, 21 January 2011.

1. Comments on the statements on slide 32

"R is much higher for Hoffmann analytes than for NFDPM "

"R is higher for low tar products"

It should be first noted that the "R" values given in the slide 32 are not reproducibility figures obtained on an agreed method but are estimates of reproducibility for participating laboratories when each was using its own preferred methodology as reported in the 2006 CORESTA study[1]. All other R values in the presentation (slide 34) were obtained from collaborative studies according to ISO 5725 as given in CORESTA Recommended Methods.

The low tar product (1R5F) data is given in blue points on slide 32 and has more variability than the higher tar product (2R4F) for all 34 analytes listed on the X axis except one analyte.

First, it should be noted that all the Hoffmann analytes were investigated in this study except trace metals which could only be performed by a minimal number of participating laboratories at that time.

Quinoline was the only analyte where the higher tar product had greater variability than the low tar product. This highlighted that its measurement method was probably quite different between different laboratories with some giving quite inaccurate data. This analyte was not considered a priority and so far no further work has been done to elucidate this effect.

For all the studied Hoffmann analytes, each laboratory was using its preferred method and so one would expect that variability would be greater than that for TNCO using standardised methods.

This highlights the value of standardized methods and demonstrates how easy it would be to compare data between laboratories that use different methods and make the wrong conclusions without the reproducibility on hand to contextualize results.

Even so, the reproducibility of Hoffmann analytes after undergoing rigorous collaborative studies, where all laboratories used the same Recommended Method, is still higher than TNCO. This will be due to various factors, each depending to a lesser or greater extent on the specific analyte. Some of the most important considerations are:

[1] Determination of Hoffmann Analytes in cigarette mainstream smoke. Beiträge 2009, 23(4), 161-202.

- The levels of the Hoffmann analytes are measured in micrograms or nanograms which are a factor of a 1000 or a million times lower than TNCO that are measured in milligrams and as a consequence increased variability occurs.
- TNCO are measured either gravimetrically for TPM; from a gas bag for CO or from a simple solvent extract followed by gas chromatography analysis for nicotine. The determination of many other analytes necessitates clean-up procedures before measurement and potential interference from other compounds in the smoke matrix and these factors are bound to have an effect on R.
- Some compounds, such as benzo[a]pyrene may not fully separate from other components during the chromatographic analysis as already discussed in Q1.
- Smoke produced from a cigarette is a dynamic and complex mixture. Certain analytes are unstable (such as 1,3-butadiene) and may need to be measured quickly after collection. Methods to collect gas phase compounds involve adding a trapping system into the system. Depending on the set-up this can increase dead volumes and introduce time lags between smoke generation and collection as well as change air flow rates or puff shape compared to those defined in the ISO standards. These factors need careful adjustment during method set up and validation.
- Connection tubing to traps has the potential to absorb some reactive compounds and any effects need to be investigated prior to use.
- The type of smoking machine, coupled with the chosen smoking regime, may have a significant effect on certain analyte yields as found in the recent collaborative study on TNCO by the ISO Working Group 10.
- Different machine types will have other differences. If cold traps are attached i.e. distance between smoke generation to the smoke collection trap. The time for smoke collection will be different between linear and rotary smokers and this may have an effect on the levels of the most reactive / unstable compounds
- Data variability will also depend on human error i.e. the experience, training and expertise of the technicians and the care and maintenance of equipment being used in laboratories. Where available, a partly or fully automated procedure may help to reduce this type of variability.
- ISO 17025 accreditation requires laboratories to take part in regular collaborative studies so that their data can be compared with other laboratories and this contributes to the minimization of variability. Actually, most if not all CORESTA participants have such accreditation or an equivalent national standard.

2. Comments on statement on slide 39

"High levels of inter-laboratory variability observed"

In many cases the variability is much higher for inter-laboratory than within a lab. This is what CORESTA learned about the cause of inter-laboratory variability and ways of reducing it, both generally and for specific methods.

CORESTA has been involved in method development for many years and as such, much of its collective learning and experience has been transferred into its recommended methods and onwards into ISO standards where applicable.

There are several general steps that are incorporated into a smoke method and control of each is recognized by the CORESTA participants to have an effect on inter-laboratory variability.

1. Smoke is produced under the prescribed ISO smoking regime.
   - Care must be taken to ensure that the cigarette conditioning and testing atmosphere are well controlled as well as setting up the smoking regime according to the relevant ISO standards for subsequent tar, nicotine and CO measurement.
   - Different levels of variability may be expected based on any different smoking regime.
2. Smoke is collected either directly onto a Cambridge filter pad (CFP) or collection in a trap situated either after the CFP or with no CFP included.
   - Introduction of trapping systems different than the CFP for other analyte measurement has the potential to affect yields (due to changes in air flow, dead volumes before collection, puff shapes). Values for these parameters are set out in the relevant ISO standards. A sufficient number of traps in series must be used to ensure high analyte recovery.
3. Other components of the smoke matrix can interfere with the measurement of other targeted smoke analytes and in some cases a smoke clean-up prior to measurement is needed.
   - Even after clean-up, some compounds, such as benzo[a]pyrene in the example given in the presentation to FDA, do not fully separate from other components during the chromatographic analysis and how that peak is integrated during measurement can have a significant effect on its level. It is not always easily explainable but not every laboratory experiences this lack of separation whereas for others the compound of interest and the interfering compound may co-elute.
   - Care must be taken concerning the purity of chemicals used as calibration standards.

Within-lab variability is a component of inter-laboratory variability and so is also an important factor for control. Further issues relating to method validation are discussed in § 6.

The CORESTA Special Analytes Sub-Group has learned that it is advisable to run a joint experiment before the main collaborative study to allow some training as well as to investigate any particular aspects of the method.

Another learning is to have realistic expectations on the level of variability that might be obtained for the Hoffmann analytes. All the analytes studied so far give considerably higher between laboratory variability than TNCO. There is no guidance in any general ISO standards regarding what is an acceptable level of measurement variability. The R value is what it is and it took the CORESTA group some time to accept that it was not possible to make step change improvements on the R values after those made in the first few collaborative studies. For example, a series of ring trials, proficiency tests and ISO collaborative studies carried out worldwide over many years have shown that the known variability around TNCO yields cannot be overcome. Even laboratories with ISO 17025 or GLP accreditation will produce "outliers" from time to time. As all measurements are and will be subject to error, processes have been established to monitor variability within and between laboratories and to agree on reasonable and realistic tolerances. These are given in ISO 8243 and are recognized, for example, within European guidelines.

The learning made by the CORESTA study groups is provided in guidance notes within many of the CORESTA Recommended Methods to help new users. CORESTA have also published papers, for example those previously provided to the FDA, on the work of the Special Analytes Sub-Group and these provide further details on specific studied factors. Some of these issues have been previously documented, for example, in Table A4 of the UK benchmark study[2].

The most efficient way of reducing uncertainty is for laboratories to seek active participation in working groups which allow open dialogue and exchange of scientific and technological know-how to avoid both misinterpretation of data and false conclusions with respect to potential product compliance.

3. Efficiency of the DNPH derivatization in the carbonyl study

Smoke represents a highly dynamic mixture, with rapidly changing properties of the smoke matrix as it elutes and condenses from a cigarette. These changes include the decrease of some constituents and the artificial formation of others at the same time. In order to make different samples of machine generated smoke comparable with respect to carbonyls, a strictly defined sample handling procedure after smoking is advisable (temperature, duration of derivatization, time until measurement and time differences between measurements). From an operational point of view, these factors must be considered as well as the issues of completion and under/overestimation with respect to derivatization.

When the Sub-Group started work on selected carbonyls the derivatization issue was discussed and was a critical part of the joint experiment carried out prior to the collaborative study (the whole process being outlined in § 6). Three factors were

[2] E Gregg et al., The UK smoke constituents testing study. Summary of results and comparison with other studies. Beiträge 2004 21(2), 117-138.

identified for specific study i.e. the volume of the trapping solution in the impinger trap; the mineral acid for the derivatization step and the completion time for the derivatization step (5 or 30 minutes). The results showed no significant increase from a 5 to a 30 minute derivatization. The trapping efficiencies of the liquid traps were found to be crucial for complete collection. Each laboratory determined whether any DNPH derivative was measured in the last of a series of traps. If none were found then the trapping efficiency of the earlier traps was considered sufficient. As long as an excess of DNPH was used and un-reacted DNPH was detected in the chromatogram then a complete reaction was assumed.

4. Accounting for carbonyls underestimation, linked to incomplete reaction

In addition to the aspects studied in and discussed following the joint experiment, laboratories had shared their experiences and data from other methods. For example, work on direct collection of the gas phase in a glass syringe provided very similar smoke yields on reference cigarettes to those found by the DNPH method[3].

5. Standards used to correct for this

Other factors concerning the derivatisation are also important. For example, when calibration standards (carbonyls) are derivatised they form only one isomeric derivative.

However, when smoke samples are derivatised in mineral acids to further push the equilibrium towards the derivative, two isomers are formed (cis and trans) and particularly for acetaldehyde the additional isomer forms a significant portion of the total and must be included in the overall quantitative measurement in smoke. The minor carbonyls may also form additional isomers but these are not easily separated from interfering components in the matrix and so have not been included in the quantification as pragmatically agreed by the SubGroup members for the Recommended Method.

During method validation, additional reference compounds (such as acetaldehyde and other carbonyls) are added at different concentrations to the collected smoke. Percentage recoveries are determined, again, ensuring that there is an excess of the derivatizing reagent. Recovery levels are compared across laboratories to further address the raised FDA question.

6. Primary factors that cause R to be increased for specific analyses

The primary factors that cause an increase in R are dependent on the studied analyte and the chosen method. Proper method validation is required with investigation and discussion by participants at each step in the process rather simply following a method taken from the literature. Different considerations need to be

[3] Studies on alternative analytical methods for the determination of organic compounds in the gas phase of mainstream cigarette smoke. M. Intorp, CORESTA Meeting 2004. ST 29.

made for each studied smoke component although there will be key validation principles that are set out in the ISO 17025 accreditation standard (e.g. determination of limits of quantification / limits of detection / recovery rates / concentration range for which the method has been calibrated / within-laboratory variability range).

CORESTA method development usually includes the following steps:

1. An evaluation of the range of available methods and equipment used in various laboratories is done firstly by collating details of the various aspects of those methodologies in a questionnaire format and tabulation of data for comparative purposes. Where work on in-house methods has produced data for reference cigarettes this information may also be shared.
2. The Sub-Group discusses the method differences and the weaknesses of certain approaches followed by broad agreement on common or well understood method components that can be harmonized and aspects that need further work.
3. A joint experiment is then organised, preferably statistically designed, to investigate the identified method components that the participants have put forward as potentially affecting incomplete measurement or data variability. A limited number of test articles, usually reference cigarettes, are used. This phase allows some training to implement the new method in-house and to generate internal validation data.
4. The Sub-Group discusses results of the joint experiment and decides whether there is a sufficient basis for harmonization and for moving forward to a full collaborative study using a recommended method. If not, the Sub-Group proposes a further joint experiment to look at other specific aspects of the methodology (followed by further discussion).
5. A collaborative study is carried out to obtain “r” and “R” values on a broad range of products with detailed guidance given to participants on conducting the study. A relatively high number of participants is required for a collaborative study with around 20 being a realistic number to obtain a robust value for R and this is perhaps uniquely only possible within CORESTA.
6. The Sub-Group discusses which guidance notes should be included in the recommended method.
7. The Sub-Group documents the work in the form of a CORESTA Recommended Method (CRM) that is made available on the CORESTA website and in many cases is published in more detail in a scientific paper.

The whole process demonstrates the value of the CORESTA forum where participants share their experiences in an open round table discussion.

A CORESTA collaborative study on volatiles has recently been finished. The yield variability with “R” expressed as a percentage of the mean yield for the 3R4F reference cigarette was 37 % (benzene) and 71 % (butadiene). Low tar products, such as the 1R5F reference cigarette, showed even higher levels. It appears difficult

to reduce the within lab variability and as such these tolerance levels will need to be formally acknowledged when it comes to comparison of test results.

Of course, if only one collaborative study is performed then there will be no indication about whether the R value improves over time as laboratories become more experienced in using the recommended methodology. Some improvements have been seen in the R values from TSNA studies over time. For example, the CRM values obtained in 2003 are lower compared with those obtained in the CORESTA 2006 study. However R values were still much higher than those obtained for TNCO.

Notes on CORESTA

Relations with ISO

As an association of tobacco related companies, CORESTA's purpose was defined by resolutions. One of these clearly stated *"that the reference systems be unified [and] analytic methods standardized".* This gave way to the CORESTA Recommended Methods (CRMs) that were tools for the industry to work in the same way and offer comparable and sound measurement results. In the same time, CORESTA member companies had delegates working in their respective standardization bodies. It came naturally that some CRMs were considered worth becoming international standards.

A New Work Item (NWI) can be proposed to the ISO by any member of a national body, and this NWI can be the integration of a CRM, where most of the work has been done and discussed and agreed upon by the very users on a worldwide scale.

When a CRM has been accepted as a NWI, a working group is formed and the standard is written, checked, voted upon and approved according to ISO procedures and templates.

As a French organization, CORESTA is a liaison member of the Tobacco Bureau of the French association for standardization (AFNOR). As for the more global TC126 which met in Rio last autumn, it is no surprise that many members of this committee belong to CORESTA member companies, but not all of them. Parts of this committee are the National delegates to the ISO TC126 global meetings, where CORESTA is also a liaison member, represented by the Secretary General and a Delegate mandated by the Board. The CORESTA delegation cannot vote, but can propose items to the agenda, and receives all the relevant information, including the progress steps from the NWI to the final ISO Standard.

CORESTA members breakdown, apart from tobacco companies.

Considering that tobacco companies are not only the manufacturers of consumer products but also the leaf growers and suppliers, the figure shown in the Washington DC meeting presentation (p.13) depicts the current distribution of CORESTA membership, including agrochemicals, equipment, paper, filter suppliers. However,

as explained when this pie was shown, some of the laboratories and research organizations belong to tobacco companies.

Discarding these, we can list the following members:

- 16 independent or government laboratories (5 in the USA)
- 3 government bodies
- 11 government research institutes
- 4 universities

It must be noted however that some of these may be directly involved with tobacco (e.g. University of Kentucky).

Programs or services for small and medium size tobacco manufacturers Relative size of CORESTA member companies.

There are no specific programs nor services (except halved membership fee) based on the size of the member organizations, whatever their activity may be. Representatives of small, medium and large companies work together with the same level of consideration in any working group or formal CORESTA body.

Out of some 180 fees paid last year, 77 came from 39 cigarette or smoking products manufacturing companies:

- 8 are multinational companies, paying fees for worldwide subsidiaries,
- 31 are independents companies (private or government owned):
  - 16 in Europe
  - 8 in Asia-Oceania
  - 5 in North America
  - 1 in Africa
  - 1 in Latin America