Commentary

Ten Years and More of 'Biosciences' in CORESTA - A Brief Historic Overview ^{*,1}

by

Wolfram Röper

Imperial Tobacco Group, Reemtsma Cigarettenfabriken GmbH, 22761 Hamburg, Germany

SUMMARY

In June 2001, the CORESTA² Board formally decided to broaden the scope of CORESTA by engaging in biosciences (other than tobacco agronomy and phytopathology), starting with *in vitro* toxicity testing and biomarkers of tobacco (smoke) exposure. Until then, work addressing biological aspects of smoking had only been done since 1996 by a special committee within CORESTA, reporting directly to the Scientific Commission, the Smoking Behaviour Committee. Membership of that committee was - similar to ACAC³ - by invitation only. The broadened scope consequently led to the re-shaping of the then Study Groups 'Smoke' and 'Technology' into 'Smoke Science' (SS) and 'Product Technology' (PT).

Subsequently, three Sub-Groups (SG) and Task Forces (TF) were set up, reflecting this change: i) SG Smoking Behaviour (name change of former Committee in 2001), ii) TF 'Nicotine Intake' (2001, later on named 'Nicotine Uptake', disbanded in 2009) and iii) TF 'In vitro Toxicity Testing of Tobacco Smoke' (2002). Finally, a new SG 'Biomarkers' was launched in 2009 with a wider scope than its predecessor TF 'Nicotine Uptake'. The work of these groups has had and still has significant impact on the scientific work within CORESTA, leading to numerous presentations at CORESTA meetings and publications in peer-reviewed journals.

This paper provides a brief analysis of some 270 presentations and posters addressing tobacco smoke toxicity, human smoking behaviour or biomarkers, delivered at CORESTA

Congresses and SSPT Joint Meetings between 1993 and 2011. More than 50% of these papers covered different aspects of toxicology, mainly *in vitro* toxicity testing methodologies, smoke exposure systems and other equipments. Other papers described the influence of cigarette design parameters on smoke toxicity. Approaches to human risk assessment were presented, including the search for suitable *in vitro* models of the major smoking related human diseases.

CORESTA began discussing smoking behaviour topics at their Vienna meeting in 1995 and received five respective presentations there; indeed, the issue has various aspects, from smoking topography and human smoke yield to smoke uptake, deposition and retention, and... Why do people smoke at all?

As early as 1996, a presentation was given on the determination of urinary mutagenicity in volunteers exposed to ETS (environmental tobacco smoke), apparently indicating a need for CORESTA to engage in this field and to face new challenges. Indeed, our knowledge of biomarkers and how to measure them has increased considerably over the years, and there is a clear trend towards using this knowledge for conducting clinical studies into the assessment of 'modified risk tobacco products'. [Beitr. Tabakforsch. Int. 25 (2013) 563–571]

¹ Presented in part at the 2012 CORESTA Congress in Sapporo, Japan (paper SS01)

² CORESTA: Cooperation Centre for Scientific Research Relative to Tobacco

³ ACAC: Agro-Chemicals Advisory Committee

Im Juni 2001 beschloss das CORESTA Board, die Aktivitäten von CORESTA - zusätzlich zur schon seit ihrer Gründung bestehenden Agronomie und Phytopathologie - auf andere Biowissenschaften wie *in vitro* Toxikologie und Biomarker der Tabakrauchexposition zu erweitern. Bis dahin wurden seit 1996 bei CORESTA Arbeiten zu biologischen Aspekten des Rauchens nur von einem sogenannten 'Smoking Behaviour Committee', das direkt an die 'Wissenschaftliche Kommission' berichtete, durchgeführt. Die Mitglieder dieses Komitees wurden - ähnlich denen des ACAC Komitees - von der 'Wissenschaftlichen Kommission' in das Gremium berufen. Das 2001 offiziell erweiterte Tätigkeitsfeld von CORESTA führte daraufhin zur Umstrukturierung der bisherigen Arbeitsgruppen (Study Groups) 'Smoke' und 'Technology' in die Gruppen 'Smoke Science' und 'Product Technology'.

Anschließend wurden drei 'Sub-Groups' (SGs) und 'Task Forces' (TFs) gegründet, die den Wechsel widerspiegeln: i) die SG 'Smoking Behaviour' (2001, entsprach dem vorherigen Komitee, das eine reguläre SG wurde), ii) die TF 'Nicotine Intake' (2001, später umbenannt in 'Nicotine Uptake' und 2009 aufgelöst) und iii) die TF 'In vitro Toxicity Testing of Tobacco Smoke' (2002). Schließlich wurde 2009, als Nachfolgerin der TF 'Nicotine Uptake' und mit erweitertem Aufgabenbereich, eine neue SG 'Biomarkers' gegründet. Die Arbeit dieser Gruppen hatte und hat wesentliche Auswirkungen auf die wissenschaftliche Arbeit bei CORESTA. Zahlreiche Präsentationen bei CORESTA und Veröffentlichungen in wissenschaftlichen Fachzeitschriften waren die Folge.

Diese Arbeit enthält eine kurze Analyse der rund 270 Präsentationen und Poster zur Toxizität des Tabakrauches, zu Raucherverhalten und Biomarkern, die auf den wissenschaftlichen Tagungen der CORESTA zwischen 1993 und 2011 vorgestellt wurden. Mehr als 50% davon behandelten verschiedene toxikologische Aspekte, überwiegend Methoden der *in vitro*-Testung, Systeme zur Exposition mit Rauch, sowie sonstige technische Ausrüstungsgegenstände für *in vitro*-Untersuchungen. Bei CORESTA begann die Diskussion von Themen zum Raucherverhalten 1995 auf einer Tagung in Wien, bei der fünf diesbezügliche Präsentationen zu hören waren. Das Thema hat sicherlich verschiedenste Seiten: von der Topografie des Rauchens über die Aufnahme des Rauches, seiner Deposition und Retention bis zu der Frage...Warum wird überhaupt geraucht?

Bereits 1996 wurde bei CORESTA über die Bestimmung der Urinmutagenität in ETS exponierten Probanden referiert, offensichtlich ein Zeichen für CORESTA, sich später auf dem Gebiet der Biomarker zu engagieren und neuen Herausforderungen zu stellen. Unser Wissen zu Biomarkern und deren Messung ist über die Jahre sicherlich beträchtlich angewachsen, und es ist deutlich erkennbar, dass dieses Wissen bei der Durchführung klinischer Studien zur Untersuchung und Bewertung von Tabakprodukten 'mit vermindertem Risiko' Anwendung findet. [Beitr. Tabakforsch. Int. 25 (2013) 563–571]

En juin 2001, le Conseil d'administration du CORESTA (Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac) a décidé formellement d'élargir le champ de recherche du CORESTA aux biosciences (en dehors des domaines Agronomie du tabac et Phytopathologie), en développant tout d'abord des tests de toxicité *in vitro* et les recherches sur l'exposition aux biomarqueurs du tabac (présents dans la fumée). Jusqu'à présent, depuis 1996, seul un comité spécial du CORESTA, chargé de transmettre directement ses résultats à la Commission Scientifique, le Smoking Behaviour Committee (Comité sur le comportement au fumage), se consacrait aux travaux relatifs aux aspects biologiques du tabagisme. De la même manière que pour l'ACAC (Comité Consultatif sur les Produits Agrochimiques), il était possible de devenir membre de ce comité sur invitation uniquement. L'élargissement du champ de nos recherches a eu pour conséquence de réorganiser les anciens Groupes d'Etudes 'Fumée' et 'Technologie' en 'Smoke Science' (SS) (Science de la fumée) et 'Product Technology' (PT) (Technologie du produit). En conséquence, trois Sous-Groupes (SG) et Equipes de Travail (ET) reflétant ces changements ont été créés; i) SG 'Smoking Behaviour' (Comportement au fumage) (modification de l'appellation du précédent comité en 2001), ii) ET 'Nicotine Intake' (Absorption de nicotine) (2001, dénommée par la suite 'Nicotine Uptake', dissoute en 2009) et iii) ET 'In vitro Toxicity Testing of Tobacco Smoke' (Test de Toxicité *in vitro* de la Fumée de Tabac) (2002). Finalement, un nouveau SG 'Biomarqueurs' a été mis en place en 2009, avec un champ de recherche élargi par rapport à son prédécesseur, l'ET 'Nicotine Uptake'. Les travaux de ces groupes ont eu et continuent d'avoir un impact significatif sur les recherches scientifiques au sein du CORESTA, et ont donné lieu à de nombreuses présentations lors de rencontres du CORESTA de même qu'à de nombreux articles qui ont reçu l'approbation des comités de lecture de revues scientifiques spécialisées.

Le présent article offre une brève analyse de quelques 270 présentations et affiches relatives à la toxicité de la fumée de tabac, au comportement tabagique des fumeurs ou aux biomarqueurs, qui ont été soumises dans le cadre des congrès ou des rencontres internationales SSPT organisés par le CORESTA, et qui se sont déroulés entre 1993 et 2011. Plus de la moitié de ces articles couvraient différents aspects de la toxicologie, principalement les méthodologies relatives aux tests de toxicité *in vitro*, les systèmes d'exposition à la fumée et d'autres équipements. D'autres articles ont exposé l'influence des paramètres de conception des cigarettes sur la toxicité de la fumée. Des approches concernant l'évaluation des risques pour l'être humain ont également été présentées, y compris concernant la recherche de modèles *in vitro* adaptés aux principales pathologies humaines liées au tabagisme.

Le CORESTA a commencé à intégrer les questions relatives au comportement tabagique lors de la conférence qui s'est tenue à Vienne en 1995. Cinq présentations sur ce thème y ont été données. Cette problématique touche en effet divers aspects, allant de la topographie du tabagisme et du rendement chez le fumeur, à l'absorption, au dépôt et à la rétention de la fumée, en passant par la question:

'Qu'est ce qui explique en fait que les gens fument?'

Dès 1996, nous a été transmis une présentation sur la détermination de la mutagénicité urinaire chez des volontaires exposés à la fumée de tabac ambiante (FTA), ce que nous avons considéré comme un signe manifeste de la nécessité pour le CORESTA de s'investir dans ce domaine et de relever de nouveaux défis. En effet, au fil des ans, nos connaissances des biomarqueurs et des techniques de quantification de ces derniers se sont considérablement développées, et l'on distingue à présent une réelle tendance à réinvestir ces connaissances destinées à mener des études cliniques dans l'évaluation des 'produits du tabac à risque modifié'. [Beitr. Tabakforsch. Int. 25 (2013) 563–571]

Introduction and historic overview

This is a very short overview of CORESTA activities related to smoke biosciences. The paper is based on the transcript of an oral presentation given at the 2012 CORESTA Congress in Sapporo, Japan. It does not intend to reflect the overall scientific developments and put CORESTA work into a broader perspective. It rather intends to demonstrate how CORESTA developed since it decided to engage in 'Biosciences'.

'Biosciences' - for the purpose of this paper - were regarded as research into smoking behaviour, biomarkers, and various toxicological issues. A search was undertaken using the latest edition of the CORESTA CD-ROM (1) (available to CORESTA members) as well as the CORESTA website (<http://www.coresta.org>), where abstracts back till 1997 can be freely accessed. To complete the literature search, all printed editions of the CORESTA 'Bulletin d'Information' covering the years before 1997 and containing meeting abstracts and still available programme booklets from individual CORESTA meetings were manually searched. When CORESTA was founded in 1956, smoke biosciences were far less developed than they are today - the predominant biological fields covered by CORESTA were phytopathology and agronomy. The first report to the US Surgeon General *Smoking and Health*, also known as the *Terry Report*, addressing health issues related to smoking, was published only in 1964 (2).

However, some research of the 'Bioscience' type was ongoing in CORESTA a long time before the organisation officially endorsed research into smoking behaviour, biomarkers of exposure and *in vitro* toxicology. At the 1958 2nd Scientific Tobacco Congress in Brussels the then French Tobacco Monopoly SEITA reported on measuring individual human puffing parameters of 83 'Gauloise' smokers, and the attempts to replicate their 'smoking profiles' on a mechanical smoking machine (3). The available abstract of this presentation mentions the parameters puff duration, puff interval, puff volume and maximum temperature reached.

Things moved on in 1995, when CORESTA decided to hold a workshop related to smoking behaviour at their Smoke-Techno meeting in Vienna. It was sort of a panel discussion with five invited presenters (4–8), and the focus was on alternative smoking regimes, and whether and to what extent current machine smoking parameters reflected human smoking behaviour. The FTC (Federal Trade Commission) smoking norm was under discussion at that

time and CORESTA decided to participate in the scientific debate.

Smoking Behaviour Committee

In 1996, a special Smoking Behaviour Committee was established, reporting directly to the Scientific Commission. Membership was by invitation only, and the members had to commit themselves to actively contribute by sharing hitherto unpublished (own) data related to human smoking behaviour. This group and some of its members were very productive in writing critical scientific reviews on e.g., compensation (9), vent blocking (10) and biomarkers of exposure (11, 12). The validity of machine smoking regimes in terms of human smokers' exposure was publicly discussed at that time.

The Committee's work clarified that - taken as a whole - compensation as well as vent blocking is incomplete, but that smokers of 'low tar' cigarettes may well smoke more intensely and inhale more nicotine than the yields, measured under current machine smoking parameters, suggest (13, 14).

Sub-Groups and Task Forces

Later, in 2001, the 'Smoking Behaviour' Committee became a regular Sub-Group of the 'Smoke Science' Study Group. The major Sub-Group achievements, some of which were reached together with the Task Force 'Nicotine Uptake', are:

- Definition of terms used for exposure to smoke (15).
- Determination of human nicotine uptake through measurement of urinary metabolites, with 'nicotine + 5' (nicotine, cotinine, trans-3'-hydroxycotinine and their glucuronides) in 24-hour urine being regarded by the Task Force as sort of a 'gold standard'.
- Investigations into 'human smoker's yield' by partial filter analysis (16–18). Other terms like yield-in-use and mouth-level-exposure were also used to describe the same thing. It was shown that this methodology well reflects actual nicotine uptake, as measured by biomarkers (16).

Finally, the CORESTA Board decided in June 2001 to broaden the scope of CORESTA, including toxicology and biomarkers of exposure. This led to re-shaping the existing Smoke & Techno Study Groups into Smoke Science and Product Technology.

Task Force 'Nicotine Intake' was set up in 2001. After publication of the definitions used for exposure to smoke (15), it was decided to re-name the TF as 'Nicotine Uptake'. The Task Force was disbanded in 2009 and replaced with a Sub-Group 'Biomarkers'.

Task Force 'In vitro Toxicity Testing' of tobacco smoke was launched in 2002. This is a very active Task Force and its achievements are noteworthy.

Task Force 'Nicotine Uptake'

The primary objective of the 'Nicotine Uptake' Task Force was to develop a recommended method. They did a lot of ring trials measuring nicotine and five of its metabolites during five years, but there was no clear trend with regard to improvement of inter-laboratory variability for individual

analytes, even though variability for the sum of metabolites, including the glucuronides, sometimes was not too bad. It was unclear whether the 'direct' or 'indirect' method should be favoured, and the group eventually decided to stop work and prepare a Technical Report for CORESTA containing all data gathered so far. The final report (19) is posted on the CORESTA website and more than 600 pages long, but it is worth reading.

Task Force 'In vitro Toxicity Testing of Tobacco Smoke'

The *in vitro* toxicity testing group started speedily. In a rather short time a report on the rationale and strategy for conducting *in vitro* testing of tobacco smoke was prepared (20). A three tier approach for testing smoke condensates was outlined in 2004, and they started to conduct inter-laboratory work to test condensates from cigarettes made from different tobacco grades or blends - employing this recommended test battery (21).

Also, they looked into different fresh whole smoke exposure systems, available to member companies, and - in my view - the results were quite surprising. Comparing the toxicity of different cigarettes employing these different exposure systems gave less different results than one might have expected (22). The reports mentioned above are available on the CORESTA website (20–22).

'Smoke Biosciences' papers delivered to CORESTA at congresses and joint meetings

It goes without saying that the topics and activities of CORESTA Sub-Groups and Task Forces were reflected in presentations given at CORESTAs annual scientific meetings. Over 50% of the 271 papers given between 1993

and 2011 were dedicated to toxicology, or had at least some reference to it. Posters were less numerous on the smoking behaviour and biomarker side than on toxicology (Table 1).

Table 1. Categorisation of the 271 'bioscience' papers and posters presented at CORESTA congresses and joint meetings between 1993 and 2011.

Category	Oral presentations (%)	Posters (%)
Toxicology	105 (39)	44 (16)
Smoking behaviour	51 (19)	8 (3)
Biomarkers	52 (19)	11 (4)
total	208	63

Developments over time

Across the years it is obvious that there already were quite a few presentations before 2001, predominantly on smoking behaviour and biomarkers. This has now changed: In more recent years, toxicology has been leading with a peak at the 2006 'anniversary' congress in Paris. Generally, congresses attracted more 'Biosciences' papers than SSPT meetings, with a peak at the 2010 Edinburgh congress. Of course, any categorisation of papers by title and abstract may be subjective, but this may be acceptable for the purpose of this paper (Fig.1).

'Smoking Behaviour and Biomarkers'

The Sub-Group 'Smoking Behaviour' and the Task Force 'Nicotine Uptake' were already mentioned above. Many

Figure 1. Smoke bioscience papers given at CORESTA meetings, across years. (C = congresses)

papers have dealt with topics related to the work of these two groups. Results confirm what was said above. Compensation surely occurs, but is incomplete. Vent blocking during a single puff cannot be complete under normal smoking conditions (14), even though staining patterns of the filter tip (23) - after having smoked the whole cigarette - may suggest something else. So, under normal conditions smokers of cigarettes with less ISO (International Organization for Standardization) "tar" will be exposed to less smoke.

Analysis of used (smoked) filters is a useful tool to estimate mouth level exposure to "tar", nicotine and possibly other analytes (16–18) and enables the study of larger groups of people, when urine or saliva sampling may be an obstacle (24).

The list of biomarkers available to assess human exposure to smoke constituents other than nicotine has grown. These include among others tobacco specific nitrosamines (25), vapour phase compounds like e.g. acrolein (26) and benzene (26, 27), but also mutagenic particulate matter, exposure to which can be assessed by measuring urinary mutagenicity (28). It has been reported that biomarkers of exposure are important tools in clinical studies for the evaluation of PREPs (potentially reduced exposure products) (29, 30).

It has been demonstrated in clinical studies that some carbon filters reduce exposure to and uptake of certain vapour phase constituents (29). It has also been reported to CORESTA that smokers of mentholated cigarettes do not take up more nicotine than regular smokers (31, 32).

To identify suitable biomarkers of effect and harm is more complicated, but they will be an essential tool to demonstrate less harm. Reduced exposure, as demonstrated by appropriate biomarkers, may not necessarily lead to reduced levels of some or all of the biomarkers of effect under study. Surely, more research is needed to clearly demonstrate that reduced exposure may eventually lead to reduced harm. This may not be easy (33). If post-market epidemiology should turn out to be the only tool, it will be impossible to use it for product development in practice.

Why do people smoke?

CORESTA never actively engaged in issues related to addiction; nevertheless, this 'hot potato' was a regular topic at CORESTA meetings.

The 8 presentations given between 1994 and 2010 (34–41) are listed below.

- 1994: The other side of smoking (34)
- 1996: Inhibition of monoamine oxidase B by cigarette smoke and its constituents (35)
- 1998: Is tobacco smoking an addiction? (36)
- 2000: Do smokers smoke simply to achieve a specific concentration of nicotine in blood? (37)
- 2003: The human psychopharmacology of nicotine (38)
- 2004: Psychopharmacology of smoking and nicotine (39)
- 2006: Some psychological determinants of tobacco use (40)
- 2010: The 'attractiveness' and 'addictiveness' of ingredients added to tobacco products (41)

They were in fact rather similar, perhaps apart from the second paper, which addresses mechanisms possibly related

to the issue (35), and from the last one (41). So John Robinson's presentation from 1994 (34) and its summary may still hold:

"[...] These scientific data offer an alternative explanation to the addiction hypothesis and argue that smokers smoke not because they are addicted, but because smokers find smoking a pleasurable activity with well-defined emotional and cognitive benefits [...]" (34).

Toxicology

Toxicology presentations and posters have been grouped into *in vitro*, *in vivo* and human risk assessment:

In vitro toxicology (87 papers)

In vivo toxicology (6 papers)

Human risk assessment strategies (11 papers)

- what are the (chemical/biological) determinants of risks associated with tobacco use?
- what is the role - if any - of single chemical analytes?
- complex mixture *vs.* (sum of) selected analytes?

For example, noteworthy presentations addressing these issues were given by GREEN and RODGMAN in 2002 (42) and MASSEY *et al.* in 2008 (43).

Numbers of *in vivo* papers were negligible, but there is obviously a growing interest regarding risk assessment. CORESTA received 11 respective presentations (e.g. 42–45), but the issue remains a controversial one. Can hazard or risk of a complex mixture be assessed or calculated by extrapolating the risks imposed by individual constituents? What about synergisms and antagonisms in smoke? Does the removal of individual potent mutagens or carcinogens from smoke lead to a reduced risk product, and how should that be demonstrated? Surely, there is a need for much more research.

In vitro toxicology

The tools of *in vitro* research are manifold, as outlined below; we have heard a lot about testing different smoke preparations, different smoke exposure systems and different biological endpoints, from bacterial mutagenicity to more complex mammalian cell systems with very specific biological endpoints, possibly related to human disease mechanisms. It will be important to select cell lines and systems fit for our specific purpose, fit for the question we want to answer. Hence, papers given at CORESTA addressed the following issues:

- Test matrices
 - smoke condensate
 - smoke extracts (whole smoke or vapour phase)
 - fresh whole smoke
- Smoking machines and exposure systems
 - submerge culture
 - air-liquid interface
- Biological endpoints
 - mutagenicity (mainly AMES assay)
 - cytotoxicity (e.g. Neutral Red Uptake)
 - genotoxicity (e.g. Micronucleus assay)
 - more specific (surrogate) endpoints possibly related to specific diseases, including the respective cell lines

The best tools are useless, or just 'nice to have', if we do not

have a vision of what we want to achieve. Just to add toxicological data as another set of data to chemical analysis may not be enough. We must be able to reasonably explain our findings, we must understand our products before we can start to modify them. First approaches to understanding the product and effects of product modifications on *in vitro* toxicity are clearly visible, as shown below (references represent a non-exhaustive selection of papers dealing with the issue):

- Effects of blends, tobacco types (46, 47)
- Effects of cigarette design modifications (48–50)
- Influence of smoking parameters (51)
- Effects of ingredients added to tobacco (52, 53)
- Effects of substances added to filters or tobacco blends that might alter the toxicity of the product (54, 55)
- Testing of PREPs and MRTPs⁴ vs. 'conventional' products (56, 57)

The challenge of how to interpret data and how to put them into context will remain, and the relevance and need of *in vitro* data as part of human risk assessment will always be discussed.

Some conclusions from in vitro research

Dark tobaccos are more mutagenic in the AMES test than bright ones (47, 49), but the reasons are unclear, total organic nitrogen affects AMES mutagenicity (48), but the known potent mutagens of “tar” may not play a significant role (58, 59). Cigarette smoke vapour phase is mutagenic as well (60), but, on a per cigarette basis, not as potent as “tar”. An increase of filter ventilation increases per mg mutagenicity of “tar”(49), more intense smoking causes the opposite (61). Vapour phase cytotoxicity increases when smoking more intensely (51). And, contrary to vapour phase cytotoxicity, which is reduced by carbon filters, there does not seem to be a simple way to significantly reduce per mg “tar” mutagenicity, even though some patents in the public domain may promise something different.

As regards general cytotoxicity the results depend on the smoke fraction and cell lines used. It is obvious that carbon filters can reduce whole smoke and vapour phase cytotoxicity (57), not necessarily “tar” cytotoxicity. And, what are the causes? Is it just the reduction of one or very few smoke constituents that have an overriding effect (62)?

Whenever we do *in vitro* research we should bear in mind that smokers are exposed to fresh whole smoke aerosol. This has to be taken into account, and at least some *in vitro* systems we use should reflect this. Also, the endpoint 'dead or live cells' may not be sufficient to describe all the effects of smoke on biological systems, that may occur at much lower levels of exposure than those leading to 50% cell mortality; perhaps, in the future, we will have to search more for endpoints possibly related to human disease mechanisms (63), and look for more subtle changes.

⁴ PREP: Potential Reduced Exposure Product; MRTP: Modified Risk Tobacco Product

Overall conclusions and outlook

During ten years of CORESTA research into smoke biosciences, CORESTA's understanding of smoking behaviour, biomarkers of exposure and *in vitro* toxicity of smoke has grown considerably. Useful methods, tools for the assessment of new products, e.g. MRTPs, have been developed, partly validated and practically applied, clinical studies included. It is hoped that CORESTA will successfully carry on with this research and continue to demonstrate scientific leadership in this area.

ACKNOWLEDGMENTS

Thanks go to numerous authors and presenters, who did biological research related to smoke science and who presented their results to CORESTA. Only their results, abstracts and full papers, respectively, made this overview possible. My apologies go to all those authors, who also contributed to the scientific debate in CORESTA and are not expressly mentioned in this paper.

REFERENCES⁵

1. CORESTA: CD-ROM Version no. 32, September 2011.
2. U.S. Department of Health, Education and Welfare, Public Health Service: Smoking and Health, Report of the Advisory Committee to the Surgeon General of the Public Health Service; PHS Publication No. 1103, Washington DC, USA, 1964.
3. S.E.I.T.A.: L'acte spécifique du fumeur; CORESTA 2nd Scientific Tobacco Congress, Brussels, Belgium, 1958, abstract 1564.
4. Reeves, N. and M. Dixon: The Measurement of Human Smoking Behaviour and the Influence of Changes in Behavioural Parameters on Mainstream Smoke Deliveries; CORESTA Joint Meeting Smoke and Technology Groups, Vienna, Austria, 1995, paper ST19.
5. Dunn, P.J., M. Youssef, and B. Bentrovato: Variations in Tar, Nicotine and Carbon Monoxide Deliveries Obtained by Smokers of the Same Brand; CORESTA Joint Meeting Smoke and Technology Groups, Vienna, Austria, 1995, paper ST20.
6. Borgerding, M.F. and L.S. Winkler: The Effect of Alternative Puffing Regimens on Relative Cigarette Performance; CORESTA Joint Meeting Smoke and Technology Groups, Vienna, Austria, 1995, paper ST21.
7. Djordjevic, M.V., J. Fan, S. Ferguson, and D. Hoffmann: The Chemical Composition of Mainstream Cigarette Smoke Generated under FTC/CORESTA and Actual Human Smoking (AHS) Conditions; CORESTA Joint Meeting Smoke and Technology Groups, Vienna, Austria, 1995, paper ST22.
8. Byrd, G.D., J.H. Robinson, W.S. Caldwell, and J.D. de Bethizy: FTC Yields and Nicotine Uptake in Smokers; CORESTA Joint Meeting Smoke and Technology Groups, Vienna, Austria, 1995, paper ST23.

⁵ Selected papers as mentioned in the text. Mentioning of any single paper does not necessarily indicate its exclusivity.

9. Scherer, G.: Smoking Behaviour and Compensation: A Review of the Literature; *Psychopharmacology* 145 (1999) 1–20.
10. Baker, R.R. and L.S. Lewis: Filter Ventilation - Has There Been a 'Cover-up'? *Rec. Adv. Tob. Sci.* 23 (1997) 152–196.
11. Scherer, G.: Carboxyhemoglobin and Thiocyanate as Biomarkers of Exposure to Carbon Monoxide and Hydrogen Cyanide in Tobacco Smoke; *Exp. Toxicol. Pathol.* 58 (2006) 101–24.
12. Tricker, A.R.: Biomarkers derived from nicotine and its metabolites: A review; *Beitr. Tabakforsch. Int.* 22 (2006) 147–175.
13. Scherer, G.: Is There Compensation When Switching to Lower Yield Cigarettes? CORESTA Joint Meeting of the Smoke and Technology Study Groups, Innsbruck, Austria, abstract IG1.
14. Baker, R.R. and L.S. Lewis: The Truth Uncovered About Filter Vent Blocking; *Information Bulletin*, CORESTA Congress, Lisbon, Portugal, 2000 p. 48, paper APST4.
15. Baker, R.R. and CORESTA Smoking Behaviour Sub Group: Seminars in Tobacco Science - Terms Used for Exposure to Smoke; *Beitr. Tabakforsch. Int.* 21 (2004) 250.
16. St.Charles, F.K., G. Krautter, D.C. Mariner, and S. Appleton: A Comparison of Human Nicotine Dose Estimates From Filter Analysis with Nicotine Metabolites Analysis; CORESTA Congress, Smoke Science and Product Technology Groups, Kyoto, Japan, 2004, paper SS7.
17. Nelson, P.R. and S. Burton: Results from CORESTA Yield-in-Use Ring Trial; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Jeju Island, South Korea, 2007, paper SSPT 46.
18. Clayton, P.M., T. Verron, and S.K. Prasad: Results of the 2010 CORESTA Part Filter Method Ring Trial; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Graz, Austria, paper ST 28.
19. CORESTA Task Force Nicotine Uptake: Final Report July 2009; available at http://www.coresta.org/Reports/Nicotine-Uptake-TF_Final-Report.pdf (accessed 08 January 2013).
20. CORESTA *In Vitro* Toxicology Task Force: The Rationale and Strategy for Conducting *in Vitro* Toxicology Testing of Tobacco Smoke (May 2004); available at http://www.coresta.org/Reports/IVT_TF_Rationale-IVT-Testing-Tob.-Smoke_Report_Jun04.pdf (accessed 09 January 2013).
21. CORESTA *In Vitro* Toxicology Task Force: Report on Interlaboratory Study of the *In Vitro* Toxicity of Particulate Matter from Four Cigarettes (Ames, Neutral Red Cytotoxicity and Micronucleus Assays); available at http://www.coresta.org/Reports/IVT_TF_Report_Part_iculate_Matter_Tox.pdf (accessed 09 January 2013).
22. CORESTA *In vitro* Toxicology Task Force: *In Vitro* Exposure of Cells to Smoke at the Air Liquid Interface; available at http://www.coresta.org/Reports/IVT_TF_Report_Smoke_Air_Liquid_Interface.pdf (accessed 09 January 2013).
23. Joza, P., W.S. Rickert, W. Wright, and M.J. Kaiserman: Factors affecting filter tip colour as a measure of mouth level exposure to tobacco smoke constituents; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups Stratford-upon-Avon, UK, 2005, paper SSPT 34.
24. Mariner, D.C., M. Ashley, G. Errington, G. Mullard, S.K. Prasad, J. Sheppard, and C.J. Shepperd: Cross-sectional Study of Cigarette Smoke Exposure in Eight Countries; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Aix-en-Provence, France, 2009, paper SSPTPOST 17.
25. Kavvadias, D., G. Scherer, M. Urban, C.J. Shepperd, F. Cheung, G. Errington, and M. McEwan: Determination of Tobacco-Specific *N*-Nitrosamines in Urine of Smokers and Non-Smokers; CORESTA Congress, Smoke Science and Product Technology Group, Shanghai, China, paper SSPT47.
26. Zhao, G., X. Yang, F. Xie, S. Wang, Q. Yan, and J. Xie: Simultaneous determination of mercapturic acids derived from benzene, 1,3-butadiene, acrolein and crotonaldehyde in human urine; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Aix-en-Provence, France, 2009, paper SSPT 07.
27. Scherer, G., K. Sterz, H.W. Hagedorn, and D. Köhler: Urinary S-Phenylmercapturic Acid (Spma) as a Biomarker for Exposure to Benzene; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Aix-en-Provence, France, 2009, paper SSPT 06.
28. Smith, C.J., D.W. Bombick, S.C. McKarns, W.T. Morgan, and D.J. Doolittle: Environmental Room Human Physiology Study Using Fresh Diluted Side-stream Cigarette Smoke; CORESTA Congress, Yokohama, Japan, 1996, paper APST2.
29. Peters, G., T. Pienemann, and W. Röper: Some Observations on Biomarker Study Design to Assess Potentially Reduced Exposure Products; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Aix-en-Provence, France, 2009, paper SSPT 09.
30. Proctor, C., C. Shepperd, A. Eldridge, K.G. McAdam, D. Mariner, and I. Meyer: Cigarette Smoke Toxicant Exposure Estimates in Smokers Switched to Reduced Toxicant Prototype Cigarettes; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Graz, Austria, 2011, paper ST 35.
31. Okada, K.: Mentholated Cigarettes and Nicotine and Carbon Monoxide Uptake - A Critical Review of the Literature; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Freiburg, Germany, 2003, paper ST 40.
32. Nelson, P.R. and P.X. Chen: Menthol smokers have lower mouth level exposure to "tar" and nicotine than non-menthol smokers; CORESTA Congress, Smoke Science and Product Technology Groups, Edinburgh, UK, 2010, paper SSPT36.
33. Ward, M.R. and E.O. Gregg: A Strategic Framework for the Assessment of Potential Reduced Exposure Products; CORESTA Congress, Smoke Science and Product Technology Groups, Edinburgh, UK, 2010, paper SSPT01.
34. Robinson, J.H., W.S. Pritchard, and P.M. Lippiello: The Other Side of Smoking; CORESTA Congress, Harare,

- Zimbabwe, 1994, paper APST3.
35. Lee, D.W., H.B. Lim, H.O. Sohn, Y.G. Lee, and U.C. Lee: Inhibition of monoamine oxidase B by cigarette smoke and its constituents; CORESTA Congress, Yokohama, Japan, 1996, paper S11.
 36. Hindmarch, I.: Is Tobacco Smoking An Addiction? CORESTA Congress, Brighton, UK, 1998, Information Bulletin, p. 22–29.
 37. Robinson, J.H., M.F. Stiles, and R.A. Davis: Do smokers smoke simply to achieve a specific concentration of nicotine in the blood? CORESTA Congress, Lisbon, Portugal, 2000, paper ST28.
 38. Sherwood, N.: The Human Psychopharmacology of Nicotine; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Freiburg, Germany, 2003, paper IG 01
 39. Warburton, D.: Psychopharmacology of Smoking and Nicotine; CORESTA Congress, Plenary Session, Kyoto, Japan, 2004, Invited Paper 1.
 40. Warburton, D.M. and N. Sherwood: Some Psychological Determinants of Tobacco Use; CORESTA Congress, Smoke Science and Product Technology Groups, Paris, France, 2006, paper SS03.
 41. Sherwood, N.: The 'Attractiveness' and 'Addictiveness' of Ingredients Added to Tobacco Products; CORESTA Congress, Plenary Session, Edinburgh, UK, 2010, paper IG01.
 42. Green, Ch.R. and A. Rodgman: Toxic Chemicals in Cigarette Mainstream Smoke - Hazard and Hoopla; CORESTA Congress, Plenary Session, New Orleans, USA, 2002, Invited Paper.
 43. Massey, E.D., C. Meredith, and F. Cunningham: Developing risk assessment paradigms for tobacco smoke constituents; CORESTA Congress, Plenary Session, Shanghai, China, 2008, paper IG02.
 44. Simmons, W.S.: Lung Cancer Risk as a Function of Cigarette Design: Filtration, Blend and Yield; CORESTA Congress, Plenary Session, New Orleans, USA, 2002, Invited Paper.
 45. Wilson, C.L., R.J. Potts, G.R. Krautter, J.A. Bodnar, M.F. Borgerding, and C.D. Garner: Development of A Risk-based Priority Toxicant List for Smokeless Tobacco Products; CORESTA Congress, Smoke Science and Product Technology Groups, Shanghai, China, 2008, paper SSPT02.
 46. Di Muro, A., C. Sorrentino, L. Del Piano, M. Abet, L. Barbato, A. Cuciniello, E. Cozzolino and P. Lombardi: Smoke Condensate Mutagenicity in Several Tobacco Types: Preliminary Study by Sister Chromatid Exchanges; CORESTA Joint Meeting Agronomy and Phytopathology Study Groups, Suzhou, China, 1999, Poster 15.
 47. Röper, W. and R. Wiecek: Strategies to Assess the Biological Properties of Tobacco Smoke; CORESTA Congress, Lisbon, Portugal, 2000, paper ST19, Information Bulletin, p.164.
 48. Doolittle D.J., D.W. Bombick, C.J. Smith, and B.R. Bombick: the Effect of Cigarette Design on *in Vitro* Toxicity; CORESTA Congress, Joint Meeting Agronomy and Phytopathology Study Groups, New Orleans, USA, 2002, paper APST5.
 49. Kalirai, K., J. Whittle, R. Cowin, A. Clack, R. Langford, and E.D. Massey: the Effect of Cigarette Design Variables on Assays of Interest to the Tobacco Industry: - 4) *In Vitro* Genotoxic Activity of Mainstream Cigarette Smoke; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups Stratford-upon-Avon, UK, 2005, paper SSPT 04.
 50. Troude, V., S. Achard, S. Destruhaut, C. Haond, and J. Sarabia: Influence of the Origin of the Cigarette Paper Cellulose on the Smoke Chemical Composition and '*In Vitro*' Toxicity Tests on Mainstream Cigarette Smoke; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Jeju Island, South Korea, 2007, paper SSPT 07.
 51. Wiecek, R. and W. Röper: The effect of puff volume on *in vitro* toxicity of mainstream cigarette smoke; CORESTA Congress, Smoke Science and Product Technology Groups, Edinburgh, UK, 2010, paper SSPT32.
 52. Massey, E.D., K.K. Kalirai, and G. Smith: *In Vitro* Genotoxicity of Smoke From Cigarettes Containing Tobacco Ingredients; CORESTA Congress, Smoke Science and Product Technology Groups, New Orleans, USA, 2002, paper ST6.
 53. Baker, R.R., E.D. Massey, and G. Smith: An Overview of the Effects of Tobacco Ingredients on Smoke Chemistry and Toxicity; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Freiburg, Germany, 2003, paper ST 16.
 54. Yao, J.H., R. Cai, H.M. Chen, and C.M. Ni: Studies on Chemistry and Mutagenicity of Cigarette Smoke Condensate From Cigarettes Containing the Natural Plant Extract AML-1 Added to the Tobacco; Bull. Spec. CORESTA Congress, Brighton, UK, 1998, paper ST22, Information Bulletin p. 170,
 55. Shin, H.J., H.O. Sohn, J.H. Han, J.Y. Hur, D.W. Lee and H.C. Hyun: Effect of Cigarette Filters on the *in Vitro* Toxicity of Mainstream Smoke; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups Stratford-upon-Avon, UK, 2005, paper SSPT 26.
 56. Doolittle, D.J., D.W. Bombick and B.R. Bombick: The Use of *in Vitro* Short Term Tests to Evaluate Progress Toward Reducing the Toxicity of Cigarette Smoke; CORESTA Joint Meeting Smoke and Technology Study Groups, Xian, China, 2001, paper ST4.
 57. Wiecek, R. and W. Röper: Comparison of *in Vitro* Smoke Toxicity of Novel Charcoal Filter Cigarettes and Benchmark Cigarettes From the UK Market; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Aix-en-Provence, France, 2009, paper SSPT 42.
 58. Fukushima, T., Y. Miura, Y. Otsu, and H. Takahashi: Contribution of Selected Smoke Constituents to TPM Mutagenic Potencies in *Salmonella typhimurium* TA98; CORESTA Congress, Smoke Science and Product Technology Groups, Paris, France, 2006, paper SS25.
 59. Fukushima, T., Y. Otsu and H. Takahashi: Contribution of Five Nitrogen-containing Compounds to TPM Mutagenic Potencies in *Salmonella typhimurium* TA98; CORESTA Congress, Smoke Science and Product Technology Groups, Shanghai, China, 2008, paper SSPT04.
 60. Wiecek, R., W. Röper, and E. Kahl: Mutagenicity of

- Cigarette Smoke Vapour Phase in the AMES Test; CORESTA Joint Meeting Smoke Science and Product Technology Study Groups, Jeju Island, South Korea, 2007, paper SSPT 42.
61. Rickert, W.S., A. Trivedi, and W. Wright: Effect of Smoking Condition and Method of Collection on TA98 and TA100 Response to Crude Smoke Condensate (CSC) From Control Cigarettes (Kentucky Reference 1R4F, 1R5F and a Canadian flue-cured monitor); CORESTA Congress, Smoke Science and Product Technology Groups, New Orleans, USA, 2002, paper ST7.
62. Röper, W., R. Wieczorek, and M. Intorp: Hoffmann Analytes and Cigarette Smoke *in Vitro* Toxicity Revisited - How Do the Data Compare? CORESTA Congress, Plenary Session, Kyoto, Japan, 2004, paper APSSPT3.
63. Breheny, D., M.D. Gaça, I.M. Fearon, G.J. Phillips, S. Faux, and K. Luettich: *in Vitro* Model Development At British American Tobacco; CORESTA Congress, Smoke Science and Product Technology Groups, Edinburgh, UK, 2010, paper SSPT33.

Corresponding author:

*Wolfram Röper
Moorreye 78
22415 Hamburg
Germany
wolframroeper@aol.com*